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Immunological Characteristics of N. Y. Strains of Influenza A Virus From the 1957 Pandemic. (24092)

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A close antigenic relationship among influenza A virus strains isolated in various parts of the world during the 1957 pandemic has been demonstrated(1), and the large differences between these strains and earlier strains have been emphasized(2,3). However, evidence that there is slight antigenic relationship between strains prevalent in 1957 and older strains has been steadily accumulating. Mulder(4) first reported the presence of antibody to the new strains, in persons over 70 years of age, and this was confirmed by Davenport(5). Patients with influenza in various locations have occasionally shown rises in antibody titer to older strains(1,2). Monovalent Asian vaccine has produced antibody response to older prototype strains(6), and polyvalent vaccine not containing an Asian strain has produced a rise in titer to Asian strains in patients over 70(7). One of the problems in detecting infection and in evaluating antibody response with Asian strains has been the low hemagglutination-inhibiting titers obtained with Japan/305/57 strain. The 1957 influenza pandemic reached its peak in N. Y. City in October. From September to November 6 strains of influenza A virus were isolated from patients at the Hospital of the Rockefeller Institute. In the present communication the immunological characteristics of these strains are described. Evidence is presented that the new strains contain an antigenic component that has been present in previous influenza A virus strains. The reactivity of viruses from the 1957 pandemic with antibody and non-specific inhibitors of hemagglutination is described. One of the new isolates is a highly sensitive indicator of the presence of hemagglutination-inhibiting and neutralizing antibodies in human serum. This property has made the Rockefeller Institute/5/57 strain especially useful in diagnosing infection with recently prevalent influenza viruses, in demonstrating antibody against them in human sera obtained before the 1957 epidemic, and in detecting an immunological response to monovalent Asian influenza vaccine.

Materials and methods. Viruses. The 1957 New York isolates were recovered in embryonated eggs by combined amniotic and allantoic inoculation of throat washings. The new strains were designated in order of isolation A/Rockefeller Institute/1/57 to A/Rockefeller Institute/6/57. The viruses were used after 2 to 4 passages in the allantoic cavity. The Far East isolates Japan/305/57 and Formosa/313/57(2) were kindly provided by Miss M. L. Miesse of the Walter Reed Army Institute of Research. The strain A/New York/1/53 was isolated at the Rockefeller Institute. The strains Swine/15, PR8, FM1, and Cuppett are well known prototype strains. *Rabbit sera.* Rabbit antisera against the 1957 isolates and Swine/15 were prepared by intravenous injection of 10 ml of virus infected allantoic fluid, followed, in 2 weeks, by intraperitoneal injection of 10 ml of the same material. The rabbits were bled 2 weeks after the second injection. Antisera against PR8, FM1, Cuppett and New York/1/53 had been previously prepared in this laboratory, and stored at 4°C. In each case a pool of sera from 2 rabbits was employed. *Patients' sera.* Acute sera and convalescent sera collected 14 to 21 days from onset of illness were obtained

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from a series of patients with the clinical picture of influenza. An additional later convalescent serum was obtained from some patients. After standing overnight at 4°C, serum was separated from coagulated blood and stored at –20°C. *Red blood cells.* Suspensions of chicken red blood cells, standardized photometrically, were used in hemagglutination and hemagglutination-inhibition titrations at a final concentration of 0.18% by packed cell volume. *Hemagglutination titrations.* Glass test tubes with an internal diameter of 10 mm and hemispherical bottoms were used. Serial 2-fold dilutions of infected allantoic fluids were made in phosphate buffered saline. To 0.5 ml of each dilution was added 0.5 ml of 0.36% chicken rbc suspension. Reactions were read after 70 minutes, and the end point was considered to be in the highest dilution showing marked but not complete agglutination (2+) of rbc. *Hemagglutination-inhibition titrations.* Human sera were inactivated at 56°C for 30 minutes. Rabbit sera, diluted with equal volumes of phosphate buffered saline, were inactivated at 65°C for 30 minutes and absorbed at 4°C with 20% chicken rbc. Both human and rabbit sera were treated(8) with *V. cholerae* filtrate to remove non-specific inhibitors of hemagglutination. Serial 2-fold dilutions of inactivated serum were made in saline. To 0.3 ml of each dilution was added 0.3 ml of infected allantoic fluid diluted so as to give a final concentration of 4 hemagglutinating units of virus in each tube. To each mixture was added 0.6 ml of 0.36% suspension of chicken rbc. Readings were made after 70 minutes at room temperature and the end point was taken as the highest dilution of serum which completely inhibited rbc agglutination. Titers are expressed as the reciprocal of the final dilution. In some titrations of human serum a lowest final dilution of 1:4 was obtained by mixing 0.6 ml of undiluted serum with 0.3 ml of diluted infected allantoic fluid, and adding 0.3 ml of 0.72% chicken rbc. *Neutralization titrations.* Human sera were inactivated at 56°C for 30 minutes prior to testing. *In ovo* neutralization titrations were carried out in 10 or 11 day old chick embryos as previously described(9), except that the inoculum of 1000 EID_{50} per egg was contained in a volume of 0.1 ml of the serum-virus mixture. *Computations.* To determine the extent of the immunological relationship among strains, serum titer ratios $\left(r = \frac{\text{heterologous titer}}{\text{homologous titer}} \right)$ were used to compute R values according to the expression, $R = \sqrt{r_1 \times r_2}$(10). The R values, multiplied by 100(9), provide estimates of immunological relationship between any 2 strains in terms of per cent. *Antibody absorption.* Virus-erythrocyte complexes were prepared according to the procedure described by Jensen and Francis(11). In absorption experiments mixtures of virus-erythrocyte complexes and *V. cholerae* filtrate treated sera were rocked for 30 minutes at a rate of 8 oscillations per minute at 5°C. The rbc were then sedimented at 1000 rpm for 10 minutes. The sera were collected and antibody content measured by hemagglutination inhibition. Experiments showed that absorption was maximal in 30 minutes.

Results. Cross hemagglutination-inhibition titrations with 1957 and prototype strains of influenza A virus and rabbit antisera. As shown in Table I, all of the 1957 isolates were antigenically closely related. Furthermore, there was evidence of slight cross-relationship between some of the 1957 strains and some of the older prototype strains. In numerous instances the relationship was evident in only one part of the cross test, but in others it was seen in both parts. The extent of immunological relationship, expressed in per cent, is shown in Table II. The close immunological relationship among all of the 1957 strains is again readily apparent. The slight but definite relationship of a 1957 isolate to an older strain is illustrated by RI/5/57† and FM1, in which case R x 100 = 2.2%. For comparison, the same degree of relatedness was found between PR8 and FM1. In all hemagglutination-inhibition titrations with immune rabbit sera, corresponding pre-immunization sera were used as controls for the presence and level of non-specific inhibitors. In a few instances non-specific inhibitory activity was

† RI = Rockefeller Inst.

TABLE I. Cross Hemagglutination-Inhibition Reactions with 1957 Isolates and Prototype Strains of Influenza A Virus.

Rabbit antiserum	Virus: Far East		Virus: New York						Virus: Prototype strain				
	Japan /305	Formosa /313	RI/1/57*	RI/2/57	RI/3/57	RI/4/57	RI/5/57	RI/6/57	Swine/15 (1931)	PR8 (1934)	FM1 (1947)	Cuppett (1950)	NY/1 (1953)
Serum hemagglutination-inhibition titer													
Japan/305/57	*2048*	512	8192	4096	4096	2048	2048	4096	<16	16	16	16	<16
Formosa/313/57	1024	*1024*	4096	2048	"	"	"	2048	"	"	32	<16	16
RI/1/57	"	256	*4096*	"	"	"	"	"	"	"	16	"	<16
2	"	"	"	*2048*	"	"	"	"	"	"	"	"	"
3	"	"	8192	4096	*4096*	"	"	"	32	32	32	"	16
4	"	"	"	"	8192	*2048*	"	4096	<16	"	"	16	32
5	256	64	4096	1024	4096	1024	*2048*	1024	32	"	64	"	"
6	512	128	"	2048	"	"	"	*2048*	<16	‡	16	"	16
Swine/15 (1931)	<16	<16	32	<16	<16	‡	32	<16	*2048*	128	32	32	32
PR8 (1934)	"	"	"	"	"	32	"	"	<16	*8192*	"	<16	"
FM1 (1947)	"	"	16	"	"	"	16	"	"	128	*1024*	512	256
Cuppett (1950)	"	"	<16	"	"	16	<16	"	"	16	4096	*4096*	512
NY/1 (1953)	"	"	"	"	"	<16	"	"	"	256	512	64	*8192*
Titer ratio†													
Japan/305	*1*†	1/4	4	2	2	1	1	2	<1/128	1/128	1/128	1/128	<1/128
Formosa/313	1	*1*	4	2	4	2	2	2	<1/64	1/64	1/32	<1/64	1/64
RI/1/57	1/4	1/16	*1*	1/2	1	1/2	1/2	1/2	<1/256	1/256	1/256	<1/256	<1/256
2	1/2	1/8	2	*1*	2	1	1	1	<1/128	1/128	1/128	<1/128	<1/128
3	1/4	1/16	2	1	*1*	1/2	1/2	1/2	1/128	1/128	1/128	<1/256	1/256
4	1/2	1/8	4	2	4	*1*	1	2	<1/128	1/64	1/64	1/128	1/64
5	1/8	1/32	2	1/2	2	1/2	*1*	1/2	1/64	1/64	1/32	1/128	1/64
6	1/4	1/16	2	1	2	1/2	1	*1*	<1/128	‡	1/128	1/128	1/128
Swine/15 (1931)	<1/128	<1/128	1/64	<1/128	1/128	‡	1/64	<1/128	*1*	1/16	1/64	1/64	1/64
PR8 (1934)	<1/512	<1/512	1/256	<1/512	<1/512	1/256	1/256	<1/512	<1/512	*1*	1/256	<1/512	1/256
FM1 (1947)	<1/64	<1/64	1/64	<1/64	<1/64	1/32	1/64	<1/64	<1/64	1/8	*1*	1/2	1/4
Cuppett (1950)	<1/256	<1/256	<1/256	<1/256	<1/256	1/256	<1/256	<1/256	<1/256	1/256	1	*1*	1/8
NY/1 (1953)	<1/512	<1/512	<1/512	<1/512	<1/512	<1/512	<1/256	<1/512	<1/512	1/32	1/16	1/128	*1*

* RI = Rockefeller Inst. † Titer ratio = heterologous titer/homologous titer. ‡ Non-specific inhibitor not completely eliminated.

TABLE II. Comparison of Extent of Immunological Relationships among 1957 Far East and New York Isolates and Prototype Strains of Influenza A Virus as Shown by the Hemagglutination-Inhibition Procedure.

Rabbit antiserum	Far East		New York						Prototype strain				
	Japan /305	Formosa /313	RI/1/57	RI/2/57	RI/3/57	RI/4/57	RI/5/57	RI/6/57	Swine/15 (1931)	PR8 (1934)	FM1 (1947)	Cuppett (1950)	NY/1 (1953)
Japan/305/57	100*												
Formosa/313/57	50	100											
RI/1/57	100	50	100										
2	"	"	"	100									
3	71	"	141	141	100								
4	"	"	"	"	141	100							
5	35	25	100	71	100	71	100						
6	71	35	"	100	"	100	71	100					
Swine/15 (1931)	<.8	<1.1	<.8	<.8	.8	†	1.6	<.8	100				
PR8 (1934)	<.4	<.6	<.4	<.4	<.4	.8	.8	†	<1.1	100			
FM1 (1947)	<1.1	<2.2	<.8	<1.1	<1.1	2.2	2.2	<1.1	<1.6	2.2	100		
Cuppett (1950)	<.6	<.8	<.4	<.6	<.4	.6	<.6	<.6	<.8	<.3	71	100	
NY1 (1953)	<.4	<.6	<.3	<.4	<.3	<.6	<.8	<.4	<.6	1.1	17	3.1	100

* Expressed as per cent of immunological relationship: $R \times 100 = 100\sqrt{r_1 \times r_2}$.

† Could not be calculated because of non-specific inhibition.

not reduced below a titer of 1:16 by the procedure used. However, residual non-specific inhibitory activity interfered with determination of antibody levels only in 2 instances, as indicated in Table I. In these cases the hemagglutination-inhibiting activity of pre- and post-immunization sera was equally low. In certain other cases, the hemagglutination-inhibiting activity of post-immunization sera was much higher (8 times or greater) than that of the pre-immunization sera, therefore it was concluded that high inhibitory activity in post-immunization sera represented antibody. These cases involved the RI/4/57 strain and rabbit sera of recent origin. Pre-immunization controls of antisera against the prototype strains lacked inhibitory activity against RI/4/57. These sera had been prepared several years earlier and had been stored at 4°C.

Hemagglutination-inhibiting antibody levels in sera from cases of influenza. Table III summarizes the results obtained with paired sera from 13 patients seen in the fall of 1957. From 6 of these patients virus was isolated. It can be seen that no patient showed antibodies against Japan/305/57 in acute phase serum and that only 5 showed a rise of low degree in convalescent serum. In marked contrast, 4 patients showed antibodies against RI/5/57 in acute phase serum and 12 showed a moderate to marked rise in convalescent serum. The titers in convalescent serum against the other 1957 isolates were higher than those against Japan/305/57, but considerably lower than those against RI/5/57. The latter was true, with one exception, even when the serum titer against the homologous isolate was compared to that against RI/5/57. Two other patients, ET and JV, with typical responses to the 1957 strains showed a rise to Swine/15 and one, MB, showed a rise to FM1. One patient, IB, age 62, was unique in showing no rise to any 1957 strain, but a considerable rise against Swine/15 and PR8, and an even greater rise against FM1.

A third serum specimen was obtained from some patients approximately 2 months from the onset of illness. On the average a 4-fold decrease from the second specimen antibody level was observed in these sera.

TABLE III. Hemagglutination-Inhibiting Antibody Rise in Patients with Influenza, Fall 1957, New York.

			Hemagglutination-inhibition titer								
			Influenza A virus strain								
			1957						1931	1934	1947
Patient	Age	Serum	Japan /305	RI/1	RI/2	RI/3	RI/5	RI/6	Swine/15	PR8	FM1
RG (RI/1/57)*	19	A	<8	<16	<8	<16	32	<16	<16	1024	2048
		C	"	*512*	64	128	2048	512	"	"	"
ET (RI/2/57)*	42	A	"	<16	<8	<16	<8	<16	32	64	16
		C	"	256	*8*	64	1024	128	256	"	"
EC (RI/3/57)*	23	A	"	<16	<8	<16	<8	<16	<8	512	512
		C	"	256	32	*128*	1024	256	"	"	"
Tuc (RI/4/57)*	24	A	"		<8		<8			4096	"
		C	16		64		2048			"	"
PC (RI/5/57)*	28	A	<8	<16	<8	<16	<8	<16	<8	1024	256
		C	"	"	"	"	*32*	"	"	"	"
LW (RI/6/57)*	21	A	"	"	"	"	32	"	<16		
		C	8	256	64	128	256	*512*	"		
VG	21	A	<8		<8		<8			512	2048
		C	"		32		256			"	"
MV	20	A	"	<16	<8	<16	<8	<16	<8	"	512
		C	16	512	128	256	8192	256	"	"	"
JV	25	A	<8	<16	<8	<16	<8	<16	<16	1024	1024
		C	32	256	64	64	1024	128	32	"	"
IB	62	A	<8		<8	<16	64	<16	<8	128	32
		C	"		"	"	"	"	128	1024	1024
MB	58	A	"		"		<8		512	"	128
		C	16		32		1024		"	"	512
SV	27	A	<8	<16	<8	<16	<8	<16	1024	8192	4096
		C	"	8192	1024	4096	32,768	16,384	"	"	"
SO	32	A	"	<16	<8	<16	16	<16	128	2048	64
		C	"	32	"	16	256	16	"	"	"

* Virus isolated.

A = acute; C = convalescent.

Results of titrations with RI/4/57 and patients' sera are not shown because attempts to eliminate non-specific inhibitors of hemagglutination for this strain were unsuccessful. With all the other strains *V. cholerae* filtrate treatment of serum was effective.

TABLE IV. Neutralizing Antibody Rise in Patients with Influenza, Fall 1957, New York.

		In ovo neutralization titer	
		1957 influenza A virus strain	
Patient's serum		RI/2	RI/5
ET (RI/2/57)*	Acute	<8	15
	Convalescent	"	53
EC (RI/3/57)*	Acute	"	<8
	Convalescent	"	64
PC (RI/5/57)*	Acute	"	<8
	Convalescent	"	29
JV	Acute	"	12
	Convalescent	20	64
SO	Acute	<8	<8
	Convalescent	"	28

* Virus isolated.

Neutralizing antibody levels in sera from cases of influenza. *In ovo* neutralization tests (Table IV) demonstrated the presence of neutralizing antibodies against RI/5/57 in acute phase serum from 2 of 5 patients, and failed to reveal any against RI/2/57. A rise in antibody titer was found in all of the 5 patients with RI/5/57 as the test virus, but only in one of the 5 with RI/2/57. These findings confirmed the results of hemagglutination-inhibition titrations.

Hemagglutination-inhibiting antibody levels in sera obtained before the 1957 epidemic. Because of the finding of antibodies to RI/5/57 in the acute phase sera of patients, an

TABLE V. Presence of Hemagglutination-Inhibiting Antibodies against 1957 Isolates and Prototype Strains of Influenza A Virus in 24 Human Sera, 1940-1957. Japan 305 (Far East) and RI/2/57 (N. Y.) are all <4.

Serum		Hemagglutination-inhibition titer — Influenza A virus strain —			
		New York	Prototype strains		
Computed present age	Year bled	RI/5/57	Swine/15 (1931)	PR8 (1934)	FM1 (1947)
72	1940	64	<8	64	8
37	"	8	<8	<16	<8
35	1942	128	<8	128	16
*	"	8	8	32	<8
45	"	4	<8	<8	<8
*	1943	8	<8	8	<8
37	"	16	128	512	8
45	"	4	16	16	<8
48	1944	4	16	32	16
29	1945	<4	<8	64	16
35	"	4	8	<8	<8
39	1947	64	16	32	32
53	1948	4	32	512	16
39	"	<4	<8	16	<8
54	"	4	<8	64	16
26	1951	<4	8	2048	512
46	1952	<4	8	128	16
48	1953	32			
71	"	16	32	512	512
40	1954	<4	8	128	16
57	1955	<4	8	256	8
35	1956	16	64	32	32
17	1957	128	<8	1024	1024
62	"	<4	<8	64	64

* Not known.

investigation of antibody levels of sera which had been obtained from 1940 until May 1957 was carried out. These individual sera were obtained from 24 patients with various diseases and had been stored at 4°C. Table V shows that in 24 sera no antibodies were demonstrated to Japan/305/57 or to RI/2/57. In contrast, 17 sera contained antibodies to RI/5/57, though in some cases the concentration was very low. The computed present ages of these patients varied from 17 to 72. There was no apparent correlation between antibody titers to RI/5/57 and those to the older prototype strains.

Antibody response to monovalent Asian influenza vaccine. The finding of high antibody levels to RI/5/57 as compared to other strains in sera of patients with influenza led to an examination, with RI/5/57, of antibody response of 3 normal adults who had received a monovalent vaccine containing 200 CCA units per ml of Japan/305/57 strain. Each had received 2 subcutaneous injections of 1 ml 3 weeks apart, and had been bled 2 weeks after the second injection. Table VI shows that in the vaccinated persons a greater response was demonstrated with RI/5/57 than with Japan/305/57 or RI/2/57. As described above, a similar observation was made in cases of influenza.

Sensitivity to non-specific inhibitors of hemagglutination. The insensitivity of Japan/305/57 to non-specific inhibitors has been described(2,3). The relative sensitivity of the 1957 New York strains to non-specific inhibitors of hemagglutination present in human and rabbit sera treated by heating alone was determined in experiments summarized in Table VII. The human serum used was obtained 2 months prior to the onset of the 1957 epidemic in New York, and after treatment with *V. cholerae* filtrate no antibodies were demonstrated in this serum to any of the 1957 strains. It was found that 3 of the 6 New York strains were insensitive, one was slightly sensitive, and 2, RI/4/57 and RI/5/57, were highly sensitive.

Antibody absorption. The low titers in pa-

TABLE VI. Hemagglutination-Inhibiting Antibody Rise in Persons Vaccinated with Formalin-Treated Influenza A Virus, Japan/305/57 Strain.

Vaccinee's serum		Hemagglutination-inhibition titer — Influenza A virus strain					
			1957		1931	1934	1947
		Japan/305	RI/2	RI/5	Swine/15	PR8	FM1
VQ	Before	<8	<8	<8	8	64	64
	After	8	8	32	"	"	"
BL	Before	<8	<8	"	<8	1024	128
	After	"	16	256	"	"	"
HS	Before	"	<8	<8	32	128	<8
	After	"	8	64	"	"	8

TABLE VII. Sensitivity of 1957 Influenza A Strains to Non-Specific Inhibitors of Hemagglutination in Human and Rabbit Serum.

Virus	Hemagglutination-inhibition titer: Human serum* lacking antibodies against 1957 strains	Normal rabbit serum†
Japan/305/57	<8	<8
RI/2/57	"	"
3	"	"
6	"	"
1	16	32
5	512	2048
4	1024	8192

* Heated at 56°C for 30 min.
† " " 65°C " " .

tients' sera against homologous isolates, as compared to the high titers against RI/5/57, suggested the possibility that these strains had stimulated production of antibody with which they could not react efficiently. Fiset and Depoux(12) showed that some strains of influenza A virus in Q phase were capable of stimulating production in ferrets of antibodies which they were unable to absorb. To test this possibility with 1957 New York strains, antibody absorption experiments were done using as the absorbing antigen the complex formed by periodate treated virus and formalin treated human erythrocytes(11). The 2-month convalescent sera of the patients from which RI/2/57 and RI/5/57 were isolated were cross absorbed with these 2 strains. It was found that RI/2/57 and RI/5/57 could each absorb completely the antibodies against both strains from either serum if a sufficient quantity of antigen was used.

Discussion. The results of the cross hemagglutination-inhibition reactions indicate that, like other later strains isolated during the 1957 pandemic(1), the New York isolates are closely related among themselves and to the Far East strains isolated earlier in the year. The cross reactions also provide evidence for an antigenic component common to the 1957 strains and older prototype strains. Further evidence on this point is provided by the finding of antibody to RI/5/57 in acute phase sera of some influenza patients, in rises in titer of a few patients to the older strains, and in the finding of antibody levels to RI/5/57 in the sera collected from 1940 to 1957.

To overcome the difficulties which have been encountered in detecting infection and in evaluating response to vaccination with Asian strains because of the low hemagglutination-inhibiting titers obtained with Japan/305/57 strain, the use of an egg-ferret-mouse-egg line of Japan/305/57 has been recommended(1). This line gives higher titers, but is sensitive to non-specific inhibitors and requires periodate treatment of sera. Others(6) have found that titers obtained with this animal line and periodate or *V. cholerae* filtrate treated sera are not significantly higher than those obtained with an egg line of Japan/305/57 and untreated sera. In the present study a strain, RI/5/57, was found, which gives high hemagglutination-inhibiting and neutralizing antibody titers with human sera. This strain has the advantage of having been passed in eggs only. As is well known, passage in the mouse lung may lead to unpredictable alterations in the antigenic characteristics of influenza virus strains(13,14,15), whereas on egg passage the antigenic properties have been found to be stable(10,15,16). It should be emphasized that treatment of human serum with *V. cholerae* filtrate eliminates non-specific hemagglutination-inhibiting activity against RI/5/57. The usefulness of RI/5/57 in diagnosing infection with the recently prevalent influenza viruses, in demonstrating antibody in human sera collected prior to the 1957 epidemic, and in detecting a response to monovalent Asian influenza vaccine has been documented here. The results obtained indicate that unless agreement is reached concerning test antigens to be used in various studies, results will not be comparable and may be misleading.

The mechanism of difference in serological reactivity of antigenically similar influenza strains is not clear. Differences in steric arrangement of antigens has been suggested(17, 18). The possibility of a masked antigen capable of stimulating antibody production but unable to combine with it in antibody absorption experiments has been described(12). This was shown not to be the case with the RI/2/57 strain by antibody absorption experiments which demonstrated that RI/2/57 virus has the capacity to absorb all of the

antibody present to itself and to RI/5/57.

Summary. Six strains of influenza A virus were isolated in New York during the 1957 pandemic. Close antigenic relationship was found among these strains and 2 Far East isolates, and slight antigenic relationship to older prototype strains was also demonstrated. One of the local isolates, RI/5/57, was found to be a very sensitive indicator of the presence of hemagglutination-inhibiting and neutralizing antibodies against the 1957 strains of influenza A virus in human serum. Antibodies to the RI/5/57 strain were demonstrated in patients of various ages obtained from 1940 to 1957. The sensitivity of the 6 New York strains to non-specific inhibitors of hemagglutination varied from apparent insensitivity in 3 strains to extreme sensitivity in 2.

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Studies on Copper Metabolism. XXV. Relationship Between Serum and Liver Copper.* (24093)

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In a previous publication from this laboratory(1), 2 infants with hypochromic anemia, hypocupremia, hypoferremia and hypoalbuminemia were described. The parenteral administration of iron was followed by prompt hematologic response and by appreciable increase in serum albumin, but not by an increase in serum copper. The hypocupremia was alleviated only by administration of copper. It was suggested that hypocupremia was secondary to a deficiency of copper which was great enough to produce hypocupremia but which was not sufficiently severe to be significant in the pathogenesis of anemia. Little is known concerning the interrelationship between dietary, serum and liver copper. It has been shown in both swine(2) and rats(3) fed diets low in copper that hypocupremia precedes development of anemia. The extent to which serum copper concentrations reflect tissue concentrations of copper in animals fed diets containing low, normal and increased amounts of copper is unknown.

This paper presents observations on correlation between serum and liver copper in rats fed diets containing low, normal and increased amounts of this element. Data on blood he-

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