

*The epidemic of Asian influenza in 1957 provided an opportunity to study the possible effects of influenzal infection on the outcome of pregnancy. The findings presented here are suggestive of an adverse effect, but the authors indicate need for further study.*

## **THE EFFECT OF ASIAN INFLUENZA ON THE OUTCOME OF PREGNANCY, BALTIMORE, 1957-1958**

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**D**URING the past four decades many publications have appeared concerning the effect of influenza on pregnancy. Whereas experimental studies tend to support the conclusion that maternal infection detrimentally affects the fetus,<sup>1,2</sup> clinical reports have both affirmed<sup>3-6</sup> and denied<sup>7-9</sup> this effect.

The reasons for these seemingly contradictory reports are not known, but some possible explanations are apparent. The methods employed may have affected the findings. Two such methods deserve mention. Some studies have been retrospective in the collection of historic and laboratory data.<sup>3,4,6</sup> In other investigations the diagnosis of influenza has depended solely on elicitation and evaluation of clinical symptoms and signs.<sup>5-7</sup> The former is generally acknowledged as probably misleading. The latter not only fails to include inapparent infections, the effect of which although not known must be considered and elucidated, but it may also include illnesses of another etiology. Neither can reliably ascertain when infection occurred during pregnancy, and this may be a determinant of conceptual damage. There is still another factor which is unavoidably common to this and many

other investigations that probably contributes to the conflicting results: the small numbers of patients included in the study and control groups.

The epidemic of influenza A<sub>2</sub> (Asian) virus infection in 1957 provided an excellent opportunity to employ prospective and serologic methods to study the effect of influenzal infection on the outcome of pregnancy. It was postulated that the effect, if any, might be manifest by an increased number of abortions, congenital malformations, premature deliveries, stillbirths, neonatal deaths, or neonatal morbidity occurring among those infants whose mothers had influenzal infections after conception and prior to delivery. The results of this prospective study in which the diagnosis of influenza was serologically confirmed is herein reported.

### **Materials and Methods**

#### **1. Clinical and Epidemiologic Observations**

Influenza Type A<sub>2</sub> virus ("Asian" strain) began to appear in Baltimore in late September, 1957, and by the end of October was widespread in the community.

Starting on October 14, 1957, and

continuing until February 1, 1958, all patients visiting the Obstetrical Prenatal Clinic of the Johns Hopkins Hospital were enrolled in the study. They were interviewed at approximately monthly intervals until they delivered. A questionnaire was completed at each visit to determine whether they had had "flu-like" illness, the date of its onset, its duration and type, inoculations of influenza vaccine, and any household exposure to "flu-like" illness.

All deliveries occurred at the Johns Hopkins Hospital. The mother and infant were carefully evaluated at the time of delivery by the obstetrician; and on admission to and discharge from the nursery a pediatrician examined each infant. In some instances additional follow-up observations were made. Autopsies of relevant cases were performed at this hospital and reviewed by the authors, as were all case histories.

## 2. Laboratory Investigations

**Serums**—Blood was drawn at approximately monthly intervals: at each prenatal visit, at delivery, and at the postpartum visit. After separation from clots, serums were stored in screw-capped glass vials at  $-20^{\circ}\text{C}$ . All were tested first for complement fixing (CF) antibody. Only those giving a negative result were tested by the hemagglutination-inhibition (HI) method. All serums from each patient were tested simultaneously for either CF or HI antibody.

**Antigen**—For both tests antigen was prepared from influenza A/Japan/305-57 seed virus (kindly supplied by Dr. Keith Jensen) by allantoic inoculation of ten-day-old embryonated hens' eggs. After 48 hours' incubation at  $37^{\circ}\text{C}$  the allantoic fluids were harvested, pooled, lightly centrifuged, and then stored in aliquots at  $-40^{\circ}\text{C}$ .

**Complement Fixation Tests**—The method employed was that of Osler as modified by Roizman.<sup>10</sup> Serums were inactivated for 30 minutes at  $56^{\circ}\text{C}$ .

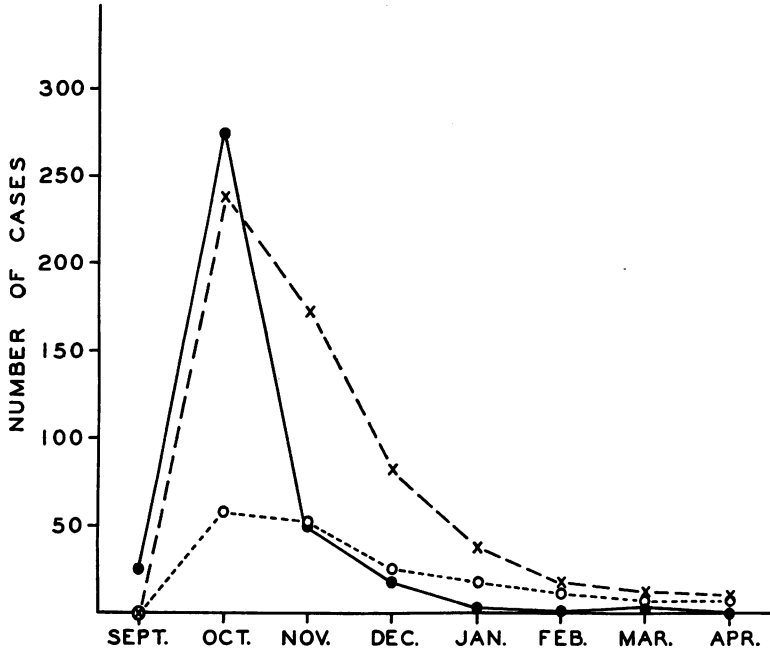
The dilution of serum to be tested (0.15 ml), complement (five units), and antigen (0.25 ml containing two units as determined by box titration) were mixed and incubated overnight at  $4^{\circ}\text{C}$ . After one-half hour at room temperature, the hemolytic system consisting of 0.1 ml equal parts, hemolysin and 5 per cent sheep RBC was added and the mixture incubated at  $37^{\circ}\text{C}$  (in a water bath) with interval shaking (every ten minutes) for one hour. The pattern was read after an additional two hours at room temperature. The titers are expressed as the highest initial serum dilution which gave more than two plus fixation.

**Hemagglutination-Inhibition Tests**—These were performed essentially as described by Jensen.<sup>11</sup> Phosphate buffered saline pH 7.2 was used as diluent. Serums were heated at  $56^{\circ}\text{C}$  for 30 minutes and treated with periodate to remove nonantibody inhibitors because the employed antigen was sensitive to these nonspecific inhibitors. Periodate treatment consisted of mixing one volume of serum with two volumes of M/90 potassium periodate and storing overnight at  $4^{\circ}\text{C}$ . Excess periodate was neutralized by adding an equal quantity of 1 per cent glycerol in saline. This procedure has been shown to satisfactorily remove these inhibitors.<sup>12</sup> Titers are expressed as the highest initial dilution of serum which produced complete inhibition of hemagglutination.

## Results

### 1. Serologic and Epidemiologic Data

Three hundred and seventy-three (56 per cent) of the 671 patients studied had a history of a "flu-like" illness. Although an average of 3.8 bloods was drawn from each of the 671 patients included in the study, since it was begun after the onset of the Asian influenza outbreak in Baltimore, relatively few patients were encountered in whose serums were demonstrated a fourfold



**Figure 1—Monthly Distribution of Clinical Illnesses and Serologic Diagnosis, “Asian” Influenza, Baltimore, 1957-1958, Woman’s Clinic, the Johns Hopkins Hospital (●—● Month During Which Cases of Clinical “Flu” Occurred; X — X Month When Single Diagnostically Significant Serum Was Obtained; o- - -o Month During Which Fourfold Rise or Fall in Antibody Titer Was Observed)**

rise in antibody titer. Accordingly, that antibody titer which for a single specimen could be said to be of probable diagnostic significance was arbitrarily defined after reference to the literature. Those titers are, for the complement fixation test, 1:20; and for the hemagglutination-inhibition test, 1:10.<sup>13-17</sup> The diagnosis of “flu” was serologically confirmed in 94 per cent of the 373 cases. Two hundred and ninety-eight individuals denied having a “flu-like” illness. However, 222 (75 per cent) of these showed serologic evidence of influenza.

Of the 671 patients included in the study, 471 (83.6 per cent) had diagnostically significant serum antibody titers as evidence of having had an Asian influenzal infection. Thirty-nine per cent of those individuals with sig-

nificant antibody levels denied having had flu-like symptoms. Twenty-four patients gave a history of “flu-like” illness but were found not to have significant antibody titers. Thus, 571 patients had clinical and/or serologic evidence of Asian influenza, an attack rate of 85 per cent. Thirty-nine per cent (222) of these were inapparent infections. Sixty-one per cent (349 individuals) of those with diagnostically significant titers gave a history of influenzal illness.

Fourfold rises were detected in the serums of 61 individuals. In 62 other individuals fourfold falls in titer were observed to occur within three months after the first specimen was obtained. The month during which influenzal infection was thought to have occurred as adjudged by a history of clinical “flu,” a single diagnostically significant sero-

**Table 1—Per cent of Clinical Category Having Particular Serologic Result**

Clinical Category	Serologic Result					Other (Negative)	Total
	CF		HI				
	1:40*	1:20	1:40*	1:20	1:10		
Clinical influenza (373 individuals)	63	13	10	5	3	6	100
No clinical influenza (298 individuals)	32	19	8	9	7	26	101

\* As routine, the highest serum dilution tested was 1:40.

logic determination, or a fourfold rise or fall in antibody titer is shown in Figure 1.

Seventy-three per cent of the cases occurred in October; 93 per cent during September, October, and November. Although arbitrary criteria had to be employed to time the infection, the resulting data are internally consistent. This time was used in association with the EDC, the birth weight, and the date of termination of pregnancy to establish when during pregnancy infection occurred.

Tables 1 and 2 show the distribution of serologic results in various categories. Data concerning vaccinated individuals are included. Only 34 persons had been vaccinated, the kind of vaccine is not known. Most of them had only one inoculation; 16 had a "flu-like" illness

**Table 2—Per cent of Serologic Result Which Occurred in Clinical Category**

Serologic Result		Clinical "Flu"	No Clinical "Flu"	Total
CF	1:40	72	28	100
	1:20	46	54	100
HI	1:40	60	40	100
	1:20	40	60	100
	1:10	37	63	100
Other (Negative)		24	76	100

and many of these were vaccinated at the time of illness. All vaccinated persons who were ill had positive serums. Three (9 per cent) of the vaccinated individuals had negative serums, none of these had had a "flu-like" illness. In tabulating the outcome of pregnancy, data concerning these vaccinated individuals were not included. Seventy-six per cent of the serologic diagnoses were determined by CF test and 24 per cent by HI test. All of the samples of serum of 15 per cent of the study population were negative by both tests.

**2. Outcome of Pregnancy in Relation to Influenza**

Twenty-seven women left Baltimore or were referred to Baltimore City Hospitals because of financial requirements, and 33 women received influenza vaccine; therefore, the results of 611 pregnancies are described.

Table 3 shows the outcome of pregnancy for 332 women who had serologically confirmed influenza, for 206 women who had inapparent infection, and for 73 women who had no evidence of influenzal infection. The incidence of abortion, stillbirth, prematurity, and congenital malformations is higher in the infected than in the uninfected group. The attack rate of 85 per cent left an uninfected population which was not large enough, however, to be a significant control group. Accordingly, the outcome of pregnancies in this hospital

**Table 3—Outcome of Pregnancy in Relation to Clinical Influenza, 611 Pregnancies: 194 White, 417 Negro Patients**

	Clinical "Flu," Serologically Confirmed, 332*		No Illness, Serologically "Positive," 206		No Illness, Serologically "Negative," 73	
	No.	%	No.	%	No.	%
Abortion	6	(1.8)	2	(0.9)	0	(0.0)
Stillbirth	8	(2.4)	2	(0.9)	1	(1.3)
Premature	27	(8.0)	20	(9.0)	4	(5.5)
Neonatal death	7	(2.1)	5	(2.4)	2	(2.7)
Neonatal morbidity	29	(8.7)	19	(9.2)	9	(12.3)
Congenital malformation	11	(3.0)	12	(5.9)	1	(1.3)

\* Number of individuals in stated category.

during the 1956-1959 period is shown for comparison in Table 4. The comparison indicates that the differences noted in Table 3 may not be significant. Further suggestion, however, that influenza infection does affect pregnancy detrimentally is obtained when the data are analyzed according to the trimester during which the infection apparently occurred (Table 5). Abortion, stillbirth, and congenital malformation occurred more frequently in individuals infected during their first trimester than those afflicted later in pregnancy or not at all. In this regard, there was no apparent difference between individuals who had a "flu-like" illness (serologically confirmed) and those who had an inapparent infection. Moreover, patients who had a severe illness, including five who were admitted to the hospital with influenzal pneumonia, did not appear to have an increased incidence of pregnancy wastage (all five hospitalized women were later delivered of normal newborns).

The kinds of anomalies found in the course of this study are listed in the footnote to Table 5. Supernumerary digits appear frequently in this Negro population, and on the basis of familial

occurrence appear to have a genetic determinant.

### Summary

The results of the serologic investigations in so far as they pertain to the immunology and epidemiology of influenza epidemics conform to the excellent and extensive data already obtained by others. A very high attack rate was experienced by the pregnant women who attended the Woman's Clinic of the Johns Hopkins Hospital. The history of clinical infection was serologically confirmed in almost all in-

**Table 4—Fetal Losses, Woman's Clinic, the Johns Hopkins Hospital**

	1956	1957	1958	1959
	Per cent			
Abortion*	3.6	4.0	3.9	6.0
Stillbirth†	2.0	2.0	2.1	2.3
Neonatal death†	2.0	2.6	2.8	2.7
Prematurity*	12.0	12.0	11.9	12.1

\* Ward patients only.

† Ward and private patients.

**Table 5—Outcome of Pregnancy in Relation to Trimester in Which Influenza Occurred**

	First Trimester 75*		Second Trimester 183		Third Trimester 275	
	No.	%	No.	%	No.	%
Abortion	6	(8.0)	3	(1.7)	0	(0.0)
Stillbirth	3	(4.0)	4	(2.1)	4	(1.4)
Premature	10	(13.0)	14	(7.7)	12	(4.3)
Neonatal death	0	(0.0)	2	(1.0)	9	(3.2)
Neonatal morbidity	16	(21.3)	17	(9.2)	30	(10.0)
Congenital malformation	8	(10.7)†	8	(4.3)‡	7	(2.5)§

\* Number of individuals in stated category.

† Three cardiac malformations (one with arthrogryposis), one twin holocardiac monster, three syndactylia, one a large area of hyperpigmentation.

‡ One hip dislocation, one malformed jaw, one multiple malformations, one malformed mouth, three supernumerary digits, one questionable mongoloid.

§ Two cardiac malformations, one club feet, one imperforate anus, one hypospadias, two supernumerary digits.

Note: These malformations were recognized at birth or in the nursery with the exception of two cases found in the review of 19 autopsy protocols, and two cases found during follow-up of 387 children.

stances, 94 per cent. Of great significance, however, is that 75 per cent of the women who denied having a "flu-like" illness had serologic evidence of infection and that 39 per cent of all infections were inapparent. This high attack rate precluded the simultaneous study of a significantly large control group. Infection seemed to adversely affect the outcome of pregnancy, especially if it occurred during the first trimester. The absence of comparative data from a true control group, however, precludes attaching too much significance to these findings. Those types of pregnancy wastage which tend to reflect adversity in the first trimester were most affected. Much more extensive data will be required to establish whether the apparently increased incidence of pregnancy wastage following first trimester influenza is real.

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#### REFERENCES

- Adams, J. M.; Heath, H. D.; Imagawa, D. T.; Jones, M. H.; and Shear, H. H. Viral Infections in the Embryo. *A.M.A. Am. J. Dis. Child.* 92:109, 1956.
- Siem, R. A.; Ly, H.; Imagawa, D. T.; and Adams, J. M. Influenza Virus Infection in Pregnant Mice. *J. Neuropath. & Exper. Neurol.* 19:125, 1960.
- Bland, P. B. Influenza in Its Relation to Pregnancy and Labor. *Am. J. Obst.* 79:184, 1919.
- Harris, J. W. Influenza Occurring in Pregnant Women. *J.A.M.A.* 72:978, 1919.
- Coffe, V., and Jessop, W. J. E. Maternal Influenza and Congenital Deformities. *Lancet* II:935, 1959.
- Saxen, Laure; Lars, Hjelt; Sjostedt, J. E.; Hakosalo, Jukka; and Hakosalo, Helja. Asian Influenza During Pregnancy and Congenital Malformation. *Acta path. et microbiol. scandinav.* 49,1:114, 1960.
- Campbell, W. A. B. Influenza in Early Pregnancy: Effects of the Fetus. *Lancet* 1:173, 1953.
- Walker, W. M., and McKee, A. P. Asian Influenza in Pregnancy, Relationship to Fetal Anomalies. *Obst. & Gynec.* 13:394-398, 1959.
- Wilson, M. G.; Heins, H. L.; Imagawa, D. T.; and Adams, J. M. Teratogenic Effects of Asian Influenza. *J.A.M.A.* 171:638, 1959.
- Roizman, B.; Hopken, W.; and Mayer, M. M. Immunochemical Studies of Poliovirus. II Kinetics of the Formation of Infections and Non-Infectious Type I Poliovirus in Three Cell Strains of Human Derivation. *J. Immunol.* 80:386-395, 1958.
- Jensen, K. E. "Influenza." In *Diagnostic Procedures for Virus and Rickettsial Diseases*. New York, N. Y.: American Public Health Association, 1956, pp. 241-263.

12. Jensen, K. E.; Dunn, F. L.; and Robinson, R. Q. Influenza, 1957, a Variant and the Pandemic. *Progress in Medical Virology* 1:165, 1958.
13. Carey, D. E.; Dunn, F. L.; Robinson, R. Q.; Jensen, K. E.; and Martin, J. D. Community-Wide Epidemic of Asian Strain Influenza, *J.A.M.A.* 167: 1459, 1958.
14. Martin, C. M.; Kunin, C. M.; Gottlieb, L. S.; and Barnes, M. W. Asian Influenza A in Boston 1957-1958. I Observations in Thirty-Two Influenza-Associated Fatal Cases. *A.M.A. Arch. Int. Med.* 103:515, 1959.
15. Hilleman, M. R.; Flatley, F. J.; Anderson, S. A.; Luecking, B. S.; and Levinson, D. J. Distribution and Significance of Asian and Other Influenza Antibodies in the Human Population. *New England J. Med.* 258:969, 1958.
16. Gresser, I., and Halstead, S. B. Serologic Response to Far East Influenza. *A.M.A. Arch. Int. Med.* 103: 590-592, 1959.
17. Widelock, D.; Klein, S.; Simonovic, O.; and Peizer, L. R. A Laboratory Analysis of the 1957-1958 Influenza Outbreak in New York City. *A.J.P.H.* 49:847, 1959.

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## APHA Medical Care Section to Hold Regional Meeting

The Regional Membership and Activities Committee of the APHA Medical Care Section extends a cordial invitation to all those able to attend an open meeting on September 12, 1961, in New York City. The proposition to be discussed at that time is: "The American people deserve the best medical care—What are we doing about it? And what should we be doing about it?"

On hand to offer some answers to these questions will be: Chairman Albert W. Snoke, M.D., director, Grace New Haven Hospital; E. M. Bluestone, M.D., consultant, Montefiore Hospital; John J. Burke, M.D., commissioner, Division of Hospital Review and Planning, New York State Department of Health; J. Douglas Colman, president, Associated Hospital Service; Henry N. Pratt, M.D., director, New York Hospital; and Martin A. Steinberg, M.D., executive director, Mt. Sinai Hospital.

The meeting will begin at 9:30 a.m. in the McMillin Theater, Columbia University, 116th Street and Broadway. No fee for registration will be charged.