Technical Papers

Identification of Another Epidemic Respiratory Disease[1]

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A mild outbreak of influenza associated with A-prime strains of virus occurred in Ann Arbor, Michigan, in the spring of 1950, and several characteristic strains were isolated. On March 20, a virus unrelated to known strains of the influenza group was isolated by amniotic inoculation of chick embryos from fresh throat garglings of a person ill on March 16. The illness experienced was similar clinically to a mild form of the influenza prevalent at the time and consisted of one day of headache and malaise, with short intervals of myalgia, but no fever was noted. Acute and convalescent blood specimens were obtained from the patient and showed rises in hemagglutination-inhibiting (HI) titer from 64 to 2,048, in complement-fixing titer from 0 to 64, and in neutralizing titer in eggs from 128 to 650. Similar tests against A, A-prime, and B strains of influenza virus showed no rises. This information clearly indicated the specificity of the virus (JJ) in relation to the illness.

In an effort to determine the relationship of this virus to the A-prime epidemic, tests were made with 52 pairs of sera that had been obtained in the acute and convalescent stages from patients among the students at the University of Michigan, ill with influenza during the epidemic phases of 1947 and 1950. None of them showed rises in titer to the JJ virus, although a majority did show typical rises in titer to strains of virus of the A-A-prime influenza group. However, it was noted that most of them did show moderately high HI titers to the JJ virus, suggesting that they had previously had experience with a virus of this type; a few of them had sufficiently high titers to suggest that their experience had been recent.

The possibility existed that the adults, because of the high titers, might not show a measurable response to infection with this virus. Attention was then directed to young children who would have less experience, and whose antibody responses would be more likely to reflect the occurrence of infection. Sera obtained from a group of children, aged 1–5½ years, who had been studied from August, 1946, to May, 1947, were available. They had been vaccinated with the PR8 strain of Type A influenza in the fall of 1946, and blood samples were obtained at intervals from August to November, 1946, and again in the latter part of April and early May, 1947 (*1*). During March and April, 1947, a sharp outbreak of A-prime influenza occurred. The serum samples from August through November could be compared with those obtained in May, 1947, for antibody rises against strains of influenza virus isolated from the epidemic. This was done and showed clear evidence of the prevalence of A-prime influenza in the Children's Home. The same sera were tested for HI antibodies against the JJ virus and, surprisingly, a large number of them showed a pronounced rise in antibodies in the sera taken in April and May, indicating that there had been a wide exposure to this virus in the interval between November and May.

Fortunately, acute and convalescent sera were also available from 11 of these children at the time of a respiratory illness in January and February, 1947. None of them presented a rise in antibodies to members of the influenza virus group or to JJ virus (Table 1). In fact, their sera were essentially devoid of antibody to the latter.

TABLE 1

TIME OF OCCURRENCE OF INFLUENZA A AND JJ VIRUS INFECTION ACCORDING TO HI TESTS

Illness	Total No.	+ A–A′	+ JJ	+ Both
Jan.–Feb., 1947	11	0	0	0
Mar.–Apr., 1947				
Adults	15	0	4	0
Children	18	11	2	2
Aug. 1946–May, 1947				
Children	68	16*	20†	32

* 13 of 16 had high antibody titers to JJ virus throughout the period of observation.

† 6 of 20 had high antibody titers to A′ throughout the period of observation.

During the influenza outbreak in March and April, pairs of sera were obtained from 33 patients, including 15 adults. Of the latter, 4 showed a rise in antibody to JJ, but none rose to influenza virus, although a majority did show high titers in the acute specimens. Of the 18 children, 11 showed sharp rises only to A-prime influenza, 2 to JJ only, and 2 to both—in contrast to the observations of January and February (Table 1). Seven other children had high titers to JJ in both the acute and convalescent sera, suggesting that they had been infected earlier with this virus.

The sera that had been obtained from 68 of the children at the end of April or early May, 1947, were then examined by HI tests in comparison with specimens from the autumn of 1946. It is seen in the last line of Table 1 that in 16 instances there were rises in titer to the A–A-prime type alone; in 20, to JJ alone; and in 32 to

[1] This investigation was conducted under the auspices of the Commission on Influenza, Armed Forces Epidemiological Board, Office of the Surgeon General, U. S. Army, Washington, D. C.

TABLE 2

SPECIFICITY OF HI TESTS WITH SERIAL SERUM SPECIMENS FROM INDIVIDUALS AND INFLUENZA A′ AND JJ VIRUSES

Case No.	Age	Virus	1946 Vaccination period		1947 Illness period				
							A′ epidemic		
			Aug.	Nov.	Jan.	Feb.	Mar.	Apr.	May
2	1 yr	A′	< 32	< 32	< 32	< 32			< 32
		JJ	32	32	< 32	< 32			512
5	1½	A′	32	1,024					512
		JJ	32	< 32		< 32			1,024
14	2	A′	< 32						1,024
		JJ	< 32						< 32
39	3½	A′	< 32	32					1,024
		JJ	< 32	< 32	< 32	< 32			< 32
17	2½	A′	< 32	< 32			< 32	512	512
		JJ	128	128			128	128	128
28	3	A′	< 32						512
		JJ	1,024						256
15	2	A′	< 32						512
		JJ	< 32						512
20	2½	A′	< 32						512
		JJ		128					4,096
P.M.	20 +	A′					< 32	< 32	
		JJ					32	4,096	

both. These data add to the evidence that the two diseases were concurrent in the population and of about the same incidence during the spring of 1947, and that they are immunologically independent.

The mean HI pre- and postepidemic titers to JJ virus in the above group were 32 and 400, respectively. Study of 30 acute and convalescent sera from patients 8–18 years of age in another institution yielded titers of 128 and 117, respectively, and, among the adult patients of 1947 and 1950, titers of 143 and 151, respectively, were found. Again the trend toward greater ease of identification of this infection in young children is illustrated.

The HI reactions obtained with the different specimens of sera of individual subjects to A-prime strains of influenza virus and to the JJ virus are listed in Table 2. They exemplify instances in which, according to HI tests, there were antibody rises to JJ alone, to both A–A-prime and JJ, or to A-prime alone, and clearly demonstrate the fact that the rises in antibody against both types of viruses occurred in the interval when influenza was epidemic. Two children with consistently high titers to JJ are included, as well as 1 adult who showed rises to JJ alone and was ill with symptoms of acute respiratory disease during the epidemic period. These results indicate the independent manner in which antibodies to the two viruses behave.

Identification and immunological relations of virus. Observation of the pecularities of the hemagglutinating property and growth characteristics of the JJ virus suggested its similarity to the strain of virus called 1233, which was isolated by Taylor (*2*) in 1947, also during an epidemic of influenza A-prime. Cross reactions with that virus and with serum obtained from Taylor demonstrated that the JJ and the 1233 strains are essentially identical. Moreover, serological tests with human sera gave closely parallel results. The 1233 strain was not being used in this laboratory during the period in which the JJ strain was isolated. The fact that both strains were isolated during epidemics of A-prime influenza suggested promptly that the new virus was related to that group of influenza viruses. The clinical histories and examinations of patients showing one or the other illness gave no clearly discriminating differences, nor was there evidence of a prevalence of any other well-recognized disease in the Children's Home at the time of the study. However, Taylor's studies with animal sera failed to show a relation to known influenza virus, to Newcastle disease virus, or to mumps virus; and his results are fully confirmed by investigations in this laboratory. Sera from mice vaccinated with the PR8 A, the FM1, or Rhodes A-prime strains had no antibodies to the JJ virus, and vice versa. Mice vaccinated with the JJ virus were not immune to the PR8, FM1, or Lee, Type B, strains. Ferrets inoculated with JJ virus had mild fever but little clinical evidence of infection. Their late sera, however, reacted equally with the JJ and 1233 strains, but not to A, A-prime, and B strains.

Because of their broader range of reaction, the extensive tests made in the present study with human sera are of still greater significance. Although 32 of the children from 1947 developed antibodies to both A-prime and JJ viruses, the remaining 36 showed specific reactions in which no cross relation between Types A, A-prime, or B influenza virus and JJ virus was detectable by HI, complement-fixation, or neutralization tests in eggs. Patients from the influenza B epidemic of 1945 (*3*) revealed no rise in antibody to the JJ virus. Moreover, subjects vaccinated against PR8, FM1, or Lee strains exhibit no rise in antibodies to the JJ virus. The virus under consideration thus seems to be serologically and immunologically distinct from previously identified strains of influenza virus.

The possibility remained, however, that it might be related to some other well-established clinical disease. Pairs of acute and convalescent sera were examined from cases of rubeola, rubella, infectious mononucleosis,[2] atypical pneumonia,[3] and the common cold,[3] with negative results. A group of sera from adults with unidentified respiratory disease, furnished by J. E. Salk, of the University of Pittsburgh, gave no positive results. Sera positive to JJ virus were negative to Newcastle disease virus. Rabbit antimumps serum, mouse sera against Melnick's Texas, Ohio, and Connecticut strains of Coxsackie virus, and mouse anti-PVM serum were all negative to the JJ virus. The egg membranes or embryos exhibit no lesions representative of the pox groups of virus. LCL bodies have not been seen, but electron microscopy of amniotic fluid has disclosed numerous filaments. There is, therefore, in these results no evidence that this virus is related to the common infectious diseases mentioned above. Hirst suggests on the basis of

[2] Sera obtained from C. J. D. Zarafonetis, of the University of Michigan.

[3] Sera obtained from A. E. Feller, of Western Reserve University.

the action of this virus upon cell receptors and egg-white inhibitor that it may be unrelated to known members of the group of hemagglutinating respiratory viruses (*4*).

Although Taylor concluded that the virus "may prove of little public health import," and Hirst noted no significant rises of antibody to it, the present data indicate that it is causally related to a widespread respiratory disease that occurs in epidemic form. Neither Taylor nor Hirst refers to the high incidence of antibodies in the adult population, which to our minds strongly indicates that the population has been thoroughly seeded. On the other hand, in young children titers are generally extremely low and, when present, tend to be quite high, suggesting recent infection. Moreover, tests with sera from as far back as 1936 indicate that the virus has been circulating since that time at least. At present, the frequency with which this virus causes clinical disease is difficult to estimate, but the epidemic herein described not only involved children but also a significant number of adults among the limited number in the institution. The clinical features in adults are not yet well outlined, but fever, cough, and coryza in the children were the common signs. The study emphasizes a frequently neglected opportunity to clarify epidemiological problems by the study of young children.

The association of the epidemic disease with influenza, the basic clinical picture, and the wide distribution of antibodies in the human population, as well as the serological and immunological characteristics of the virus, readily invite consideration of the name "Influenza C." Further studies, a number of which are under way, will determine the appropriateness of this suggestion.

References

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Kinetic Mechanisms and Hydrocarbon Flame Spectra

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It is now known (*2, 3, 6, 7, 15*) that when hydrocarbons such as C_2H_2, C_2H_4, and CH_4 burn in oxygen or air under various conditions of fuel-oxygen ratio, the flame spectrum exhibits the electronic band systems of O_2, OH, CH, C_2, as well as the hydrocarbon flame bands (2,500–4,100 A) originally obtained by Vaidya and attributed to the HCO radical.[2] The question of the manner of formation of the excited molecules and radicals in hydrocarbon flames has been a matter of considerable discussion (*2, 3*), but it has by no means been settled. The object of the present note is to report some new spectroscopic findings and to suggest kinetic mechanisms that might account for the main types of reactions that occur. The discussion is chiefly confined to the burning of acetylene in oxygen, but will be extended to other hydrocarbons in later papers.

If one designates the oxygen-fuel ratio by ρ, with $\rho = \rho_s$ for stoichiometric proportions, the results of the spectroscopic studies for the acetylene-oxygen flame may be summarized as follows (*2, 3, 6, 7*): When $\rho = \rho_s$, one finds from the inner cone the band systems of OH, CH; the Mulliken, Deslandres-D'Azambuja, Phillips, and Swan systems of C_2; the CO Fourth Positive bands; the carbon line at 2,479 A and the hydrocarbon flame bands weakly. When $\rho < \rho_s$, the Fox-Herzberg bands of C_2 also become prominent, but the hydrocarbon flame bands are very weak, if present at all. When $\rho > \rho_s$, the OH and CH bands remain strong, the bands of C_2 become very weak, the Schumann-Runge bands of O_2 increase in intensity as the oxygen concentration rises, and the hydrocarbon flame bands are enhanced.

Noting that the hydrocarbon flame bands are strongest when burning with air, with excess oxygen, or as a cool flame (*2, 3*), we have used both argon and CO_2 as diluents. The introduction of argon into the acetylene-oxygen mixture enhances the hydrocarbon flame bands somewhat, but the effect of CO_2 is particularly striking. Burning $C_2H_2 + O_2 + CO_2$ in volume proportions of about 1 : 10 : 10, and with high mass flow from a multiple-jet burner operating in air, one finds the hydrocarbon flame bands to be the most prominent feature of the spectrum (extending from $\sim$ 2,350–$\sim$ 4,100 A), the OH and CH bands to be quite weak, the C_2 bands very weak, and on the long wavelength side of the hydrocarbon flame bands, one finds weak CO flame bands. With a Bausch & Lomb medium quartz spectrograph using a 30 μ slit and type II-O Eastman plates, we have obtained well-exposed spectrograms of the hydrocarbon flame bands in the region 3,000–4,000 A in as short a time as about 30 sec. It is also interesting to note that the CO flame bands are very well developed in the oxyhydrogen flame to which is added CO_2 in rather high concentration.

It would appear that these facts may be explained qualitatively along the following lines. Oxygen is known to interact with unsaturated hydrocarbons by two distinct mechanisms (see, for example, ref. [*1*]), which may be referred to as the addition and peroxide mechanisms. In the case of acetylene the addition mechanism involves such steps as the following:

$$(1)\quad O_2 + C_2H_2 \rightarrow H{-}C(-O{-}O){-}C{-}H \rightarrow 2CHO + \sim 55 \text{ kcal}$$
$$(2)\quad HCO \rightarrow H + CO - \sim 20 \text{ kcal}$$
$$(3)\quad O_2 + HCO \rightarrow HO_2 + CO + \sim 40 \text{ kcal}$$
$$(4)\quad CO + \tfrac{1}{2} O_2 \rightarrow CO_2 \text{ (via chain mechanism)}$$

Since reaction (1) evolves about 55 kcal of energy, it is clear that, with the aid of thermal energy, it is possible for HCO to be formed occasionally in an electronically

[1] A portion of this work was supported by the U. S. Navy Bureau of Ordnance, Contract NOrd-7386.

[2] In our opinion there is as yet no unequivocal spectroscopic evidence that the emitter is HCO; however, we tend to favor this identification, as our kinetic mechanism makes it seem likely that HCO is produced exothermically.