INFECTION WITH SENDAI VIRUS IN AN OUTBREAK OF RESPIRATORY ILLNESS

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In February, 1956, an outbreak of respiratory infection, suspected to be due to an influenza virus, occurred in a hospital for the chronic sick and infirm of both sexes (many of limited intelligence). A small section of the establishment also contained rooms for the destitute. In the next month, among 180 patients, 67 cases (25 male, 42 female) were recognized, of which 21 proved fatal. The staff consisted of two part-time doctors, a matron, and 65 full-time and part-time nursing staff, none of whom had an attack of respiratory infection other than minor colds during this period. Specimens of serum were sent to the laboratory for confirmation of influenza virus infection, but no material was collected for virus isolation.

At the same time there were cases of clinical influenza among residents of the town and among nurses at the local cottage hospital. In several of these the diagnosis was confirmed as influenza virus A infection by complement-fixation (C.F.) tests with their sera. When the sera from 41 patients at the "chronic sick" hospital were examined (38 pairs, 3 single convalescent) by C.F. tests and haemagglutination-inhibition (H.A.I.) tests, only 13 of 38 (34%) had rising titres (9 of them fourfold or greater) suggestive of recent infection with influenza virus A. All gave negative results in the streptococcus M.G. agglutination test and in routine C.F. tests with influenza B and C, psittacosis, adenovirus, and *Rickettsia burneti* antigens.

At this time a strain of Sendai virus became available at the Virus Reference Laboratory. This virus, said to be a cause of pneumonitis in infants, was isolated originally in Japan. Complement-fixing and haemagglutinating antigens had been prepared to test sera from infants suffering from acute bronchiolitis with spasm in several outbreaks in the winter of 1955-6. None of these tests had provided evidence of Sendai virus infection, but interesting results were obtained when the same antigens were used to investigate the outbreak in the hospital for chronic sick.

Materials and Methods

The strain of Sendai virus, W.I.C. 12827, was kindly supplied by Dr. Isaacs, of the National Institute for Medical Research, Mill Hill; it had come originally from Dr. Ackermann, School of Public Health, Ann Arbor, Michigan. The strain had had 12 mouse, one rat, two mouse, and 14 egg passages; it had undergone two further egg passages at the Virus Reference Laboratory. The complement-fixing and haemagglutinating antigens consisted of undiluted allantoic fluid derived from 10-day-old fertile hens' eggs inoculated allantoically with 0.1 ml. of a 10^{-4} dilution of stock seed virus. After incubation for three days at 95° F. (35° C.) the

eggs were chilled overnight. Fluids containing haemagglutinating virus were pooled, and clarified by centrifugation at low speed.

The strain of mumps virus used in the course of the investigation was that of Enders, E63/2, which had had many egg passages. Haemagglutinating and "virus particle" complement-fixing antigens were prepared by the allantoic inoculation of 8-day-old fertile hens' eggs with 0.1 ml. of a 10^{-2} dilution of stock seed virus. The eggs were incubated at 95° F. (35° C.) for five to six days. They were then chilled and the allantoic fluids were harvested from those eggs containing haemagglutinating virus in high titre. The pooled fluids were spun at 20,000 r.p.m. for one hour after low-speed clarification, and the deposit was resuspended in normal saline in one-tenth the volume of the harvested fluids.

Sodium azide was added as a preservative (in a final concentration of 0.08%) to both mumps and Sendai complement-fixing and haemagglutinating antigens. The optimal dilutions of the antigens for use in the C.F. tests were determined by means of chequerboard titrations with known positive sera.

C.F. tests were carried out in "perspex" plates with cups, using 0.1-ml. quantities of inactivated test serum, complement, and antigen; $2\frac{1}{2}$ M.H.D. of complement were used and fixation of reagents took place for $1\frac{1}{2}$ hours at 37° C. Then 0.2 ml. of 1% sensitized sheep red cells was added and lysis was allowed to occur for a further half-hour at 37° C. Suitable controls were put up with the test, and all results were read next morning after standing at 4° C. overnight.

Sera tested for haemagglutination-inhibiting antibody were first treated for 18 hours at 37° C. with receptor-destroying enzyme (R.D.E.), and after inactivation at 56°-58° C. for one hour were adsorbed in the cold with a suspension of fowl red blood cells which were subsequently removed by low-speed centrifugation.

Haemagglutination-inhibition titrations were carried out in W.H.O. plastic haemagglutination plates (World Health Organization, 1953) employing 0.2-ml. doubling dilutions of the prepared sera in normal saline. Then 0.02 ml. of virus suspension (containing four agglutinating doses of virus) and 0.02 ml. of 7% fowl red blood cells were added to each serum dilution, and the results were read after mixing and standing for 30 minutes at room temperature. Suitable controls were set up with each series of tests.

Results

In consequence of the small number of patients at the hospital providing results suggestive of recent infection with influenza virus A it was decided to retest their sera with a number of other virus antigens. On testing the sera of those

TABLE I.—Results of Complement-fixation and Haemagglutination-inhibition Tests on Patients with Rising Titres to Sendai Virus

Patient	Complement-fixation Test									Haemagglutination-inhibition Test		
	Influenza A Sol. Antigen			Sendai Antigen			Mumps V Antigen			Sendai Virus		
	1	2	3	1	2	3	1	2	3	1	2	3
Ba ..	4*	4	<8	8	64	<8	8	8	<8	36	288	216
Be ..	—	<8	<8	<8	32	32	—	<8	<8	27	576	216
Sa ..	8	4	<8	<8	16	<8	<8	<8	<8	9	144	144
Hi ..	—	<8	<8	<1	8	<8	—	<1	<8	27	432	—
Ma ..	8	4	8	8	64	16	<8	<8	<8	144	—	>576
Tec. K.	—	<8	<8	<1	16	16	4	4	<8	36	—	432

* Reciprocal of the dilution of sera at which test was positive. In some instances the small quantities of sera available prevented testing at lower dilution than that stated.
— Indicates not tested.

elderly patients serologically negative for influenza virus A for their ability to fix complement with Sendai virus preparations, recent infections with this agent were suggested when five patients were found to have rising antibody titres (Table I); two other patients gave positive results at a dilution of 1:16 in both specimens (patients H and F,

Table II). In addition, the laboratory technician (patient Tec. K) who had collected blood from patient Ba suffered later from a mild respiratory illness and his sera showed rising antibody titres with Sendai virus only. Further examination by C.F. and H.A.I. tests of all remaining sera available from the hospital inmates, and other patients from the town, showed that in all probability a number had been infected recently with Sendai virus, including some who had suffered in addition from an influenza virus A infection. Table II shows those patients with elevated but unchanging antibody titres to Sendai virus, including some with rising antibody titres to influenza virus A.

Examination of further samples of sera (third specimens) collected about three months later confirmed this opinion. The Sendai virus complement-fixing antigen, which had the same constitution as the haemagglutinating antigen—that is, both were undiluted allantoic fluid—gave reproducible results from day to day with sera collected for the investi-

TABLE II.—Results of Complement-fixation and Haemagglutination-inhibition Tests in Patients Showing Non-rising Titres to Sendai from Hospital and Town

Patient	Complement-fixation Test									Haemagglutination-inhibition Test		
	Influenza A Sol. Antigen			Sendai Antigen			Mumps V Antigen			Sendai Virus		
	1	2	3	1	2	3	1	2	3	1	2	3
G (H)	<4	16	8	16	16	16	—	<8	<8	108	54	216
K (H)	8	32	16	8	8	<8	—	<8	<8	13.5	13.5	27
H (H)	<8	<8	<8	16	16	16	—	<8	<8	432	>576	—
F (H)	<8	<8	<8	16	16	<8	<8	<8	<8	27	108	—
R (H)	<4	<4	16	—	<8	8	—	<8	<8	27	27	54
Ha (T)	<4	<4	—	8	8	—	—	<8	—	144	108	—
L (T)	<2	4	<8	8	8	16	8	8	8	54	108	54
B (T)	<4	8	—	8	8	—	—	<8	—	432	432	—

(H)=hospital; (T)=town.

Sera 1 and 2 were examined in parallel in the C.F. test, but 3 was examined separately owing to shortage of sera. All three were examined in parallel in the H.A.I. test.

gation. In addition, rising titres of haemagglutination-inhibiting antibody always appeared as such on repetition of the tests, but the actual titres obtained tended to vary somewhat when the titrations were repeated on different occasions. This rendered impossible the determination of an H.A.I. baseline value for the detection of patients who had suffered from a previous infection, and from whom only one serum was available or whose paired sera showed unchanging antibody titres. It was not possible to determine exactly the times at which Sendai virus infection started and stopped in the hospital, but the first specimen from Ba, apparently the first case, taken supposedly on the third day of illness, January 20, was already positive at a dilution of 1:8; the sera collected in June (third specimens) from 29 patients providing negative results in February showed no evidence of further rises in antibody titre, with the exception of patient R, whose titre in the third specimen may not be significant because it could not be tested at the same time as the previous specimen. R was also the only patient in whom there was evidence of influenza A virus infection between the first week in March and the third bleeding in June (Table II).

The serum of one of the medical officers, collected in June, did not contain any complement-fixing antibodies at a dilution of 1:8 for influenza virus A antigen, but gave positive results at this dilution with both Sendai virus and mumps "virus particle" antigens. The significance of these findings cannot be interpreted at the moment (see below). Too few sera were obtained from the nursing staff to be able to determine the presence of infection among them.

The sera of 8 of 49 (16%) blood-transfusion donors from the town, collected in June, had Sendai virus complement-fixing titres of 1:8 or higher (five at 1:8 and three at 1:16), suggesting the possibility of infections with this virus in the local population.

While this work was in progress Dr. G. K. Hirst, of New York, visited the laboratory and reminded us that Sendai virus was similar in some respects to Newcastle disease virus. Although Jenson *et al.* (1955) found no serological cross-reactions between Sendai virus and the virus of mumps, the patients' sera were tested for antibody with mumps complement-fixing "virus particle" (V) antigen, which has an antigenic relationship to Newcastle disease virus (Jungherr *et al.*, 1949). It was found that of the six patients in whom rising Sendai virus complement-fixing titres could be demonstrated the sera of five had no complement-fixing antibody at a dilution of 1:8 when tested with mumps V antigen. The sixth patient had an unchanging titre of mumps V antibody of 1:8 in the first two specimens (Table I). The sera of five elderly patients (G, K, H, F, and R, Table II) showing unchanging Sendai virus complement-fixing antibody titres of 1:8 or higher were tested with mumps V antigen, and complement-fixing antibody was found to be absent at a dilution of 1:8.

None of the sera which gave negative results at a dilution of 1:8 in the Sendai virus complement-fixing test (including those with high haemagglutination-inhibiting antibody titres) had mumps V complement-fixing antibody at this dilution. The sera of four patients from the hospital for whom rising Sendai virus haemagglutination-inhibiting and complement-fixing antibody titres could be demonstrated gave negative results when retested with mumps V antigen for haemagglutination-inhibiting antibody.

Among 800 patients with illnesses of all descriptions from whom pairs of sera were received in 1956, five patients were found whose sera showed fourfold or greater increases in complement-fixing titre with Sendai virus and negative (titres <1:8) or non-rising complement-fixing titres with mumps "virus particle" antigen. The sera of these five patients gave negative results when examined routinely for antibodies to influenza viruses A, B, and C, psittacosis, adenovirus, *R. burnetii*, lymphocytic choriomeningitis virus, and streptococcus M.G. One of the patients was an adult with an influenza-like illness, and four were children with respiratory infections.

Discussion

In 1953 Kuroya and Ishida described the isolation of a virus from the lungs of a fatal case of pneumonitis in an infant by intranasal inoculation of mice. This virus was isolated subsequently by other Japanese workers from different laboratory stocks of mice, hamsters, and swine. Subsequent investigations (on volunteers) by Japanese workers confirmed that the virus could cause severe pneumonitis in children.

Jensen and his colleagues (1955) found serological evidence of the presence of the virus in the U.S.A. Five cases were identified, but no isolations of virus were made. The results of our preliminary serological surveys of single sera suggest that infection with the virus may not be uncommon in England, but more work is needed to ascertain the significance of these tests. The precise relationship between mumps V and Sendai antigens is being investigated at the moment. It is for this reason that all our suspected cases have been tested with mumps V antigen.

It is of interest that the recent addition to the myxo group of viruses, the "croup agent," isolated by Chanock (1956) in Cincinnati, U.S.A., and by Beale *et al.* (1956) in Toronto, also gives some serological cross-reactions with the mumps virus, but not with Sendai virus.

Summary

In an outbreak of respiratory illness in a hospital for the chronic sick, six patients (five old people and one technician) who developed rising titres of complement-fixing antibody to Sendai virus were observed.

Preliminary surveys show that antibody to Sendai virus is not uncommon in Southern England.

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REFERENCES

- Beale, A. J., McLeod, D. L., Stackiw, W., and Rhodes, A. J. (1956). Paper presented at annual meeting, Society of Pediatric Research, Buck Hill Falls, Pa.
 Chanock, R. M. (1956). *J. exp. Med.*, **104**, 555.
 Jensen, K. E., Minuse, E., and Ackermann, W. W. (1955). *J. Immunol.*, **75**, 71.
 Jungherr, E., Luginbuhl, R. E., and Kilham, L. (1949). *Science*, **110**, 333.
 Kuroya, M., and Ishida, N. (1953). *Yokohama med. Bull.*, **4**, 217.
 World Health Organization (1953). *Tech. Rep. Ser.*, No. 64. Geneva.

SUXAMETHONIUM CHLORIDE ADMINISTRATION AND POST- OPERATIVE MUSCLE PAIN

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A great deal has been written in praise of suxamethonium chloride ("scoline") as a muscle relaxant in clinical anaesthesia, stressing its freedom from side-effects such as bronchospasm, tachycardia, and fall in blood pressure. In many ways suxamethonium is the ideal relaxant: the rapid onset of complete relaxation followed by the quick return of full muscle power has made it very popular for endotracheal intubation, orthopaedic manipulation, and short abdominal operations. The ideal conditions produced for intubation have undoubtedly reduced the incidence of minor trauma to lips, tongue, and teeth, and of sore throat. One of its principal disadvantages, that of prolonged apnoea, has received so much attention as to obscure other side-effects which produce considerable anaesthetic morbidity.

It is the purpose of this paper to call attention to muscle pain and stiffness, in some cases of great severity, following the use of suxamethonium in patients who are discharged from hospital within 48 hours of operation.

Churchill-Davidson (1954) first drew attention to muscle pains after suxamethonium administration, but this received little attention in the literature; some writers denied that it even occurred. It has been noted that patients kept in bed for two to four days after operation rarely complain of post-operative muscle pains. In the present investigations dental patients discharged the day after operation were chosen. The investigations were designed to show the incidence of post-operative muscle pain, to make an estimate of its severity, and to try to find some simple means of preventing it.

Technique

The patients were premedicated with "omnupon" and scopolamine, or morphine and atropine, in doses appro-

priate to their age, weight, and physical condition. Induction was with 0.5-0.75 g. of thiopentone 5%, immediately followed by suxamethonium, 50-75 mg., and naso-tracheal intubation. Maintenance was with nitrous oxide, oxygen, and trichlorethylene. On the Nosworthy card was recorded the occupation of the patient, the dose of suxamethonium, and its speed of administration, divided into fast, medium, and slow. The resulting fasciculations were recorded as strong, moderate, and weak, and any particular muscle group strongly affected was noted. The patient's shoulders, chest, abdomen, and legs were bared in order to observe this accurately. The patients were seen the day after operation, when they were discharged, and five days later in the out-patient department, when they attended for routine dental follow-up. The site of any muscle pain or stiffness, its severity, and any spontaneous observations of the patient were recorded.

Control Series

In all, 73 patients were anaesthetized in the way described and followed up. The results were: 25 (35%) complained of pain and/or stiffness on day of discharge; 52 (72%) complained of pain and/or stiffness when seen in out-patient department; and 16 (22%) complained of severe pain and/or stiffness.

The relationship of pain and stiffness to the degree of fasciculations was studied. There seems to be no clear-cut correlation between strong fasciculation and the development of severe pain and stiffness afterwards. Some cases in which the fasciculations were weak developed severe pain and stiffness. One case is of particular interest in this connexion. The only visible fasciculation was an isolated jerk of the rectus muscle, and the patient complained post-operatively that she felt as if she had been "kicked in the belly by a horse." However, when seen in the out-patient department five days later she had experienced generalized aching for three days after discharge. Likewise, there seemed to be no definite relationship between the occupation and the state of physical fitness of the patient. Many athletic patients developed pain, and a blacksmith's striker complained of severe shoulder pain.

Our results are thus in close agreement with Churchill-Davidson's figures—that is, 72% of cases developed pain and stiffness. Our incidence of severe pain was, however, much lower: 22% as compared with 42% approximately. This difference can be explained on the basis that Churchill-Davidson's cases were out-patients, immediately ambulant after operation, whereas ours were in bed for 24 hours after operation. The longer the patient remains in bed after operation the less likely he is to develop suxamethonium pain. One patient, an architect, who had undergone five dental operations before this investigation and developed suxamethonium pain after each one, gave a very interesting account of this phenomenon. He had observed that if he kept still and made no sudden movements he was free of pain; but any activity, such as getting up out of a chair, was painful. Patients quiescent in bed after operation may not notice any discomfort. The figures from the control series show this clearly. Many patients who have no pain on the day of discharge develop it later when they return home. The incidence of pain in patients seen in the out-patient department is twice that on the day of discharge.

Experiments with Modified Technique

The following modifications of anaesthetic technique were made in an attempt to abolish muscle pains:

1. *Chlorpromazine Hydrochloride Experiment.*—Chlorpromazine hydrochloride has a direct paralytic action on skeletal muscle (Burn, 1954). It was hoped that it might modify the painful after-effects of suxamethonium. Chlorpromazine hydrochloride, 50 mg., was given intramuscularly with the premedication. A further 25 mg. was given intravenously on arrival in the theatre, followed by 0.25 g. of thiopentone, 50-75 mg. of suxamethonium, nitrous oxide, oxygen, and trichlorethylene. Fourteen cases were anaesthetized. The results were: 7 (50%) complained of