The use of transportable single-radial-diffusion immunoplates in seroepidemiological studies of influenza in the Gambia*

The occurrence and persistence of antibody to influenza A/Hong Kong/68 (H3N2) virus in selected inhabitants of two rural villages

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Seroepidemiological studies of influenza in the Gambia were made using transportable single-radial-diffusion immunoplates containing A/Hong Kong/68 (H3N2) virus as antigen. The frequency and durability of antibody so detected in selected residents of two Gambian villages (Manduar and Kafuta) are described. Transportable immunoplates were found to be an effective method for the serological surveillance of influenza and to be applicable in studies in remote areas where laboratory facilities may not be available. Results indicated that infection with influenza was widespread in Manduar residents on several occasions between 1968 and 1974 and that reinfection with A/Hong Kong/68 virus or its antigenic variants occurred frequently. Serum levels of antibodies to the haemagglutinin and neuraminidase antigens of the A/Hong Kong/68 virus often persisted for only a short time (mean half-life about 28 days), particularly after first infections. Antibody persistence increased following repeated reinfection. No precise explanation can be offered at present for the relatively short persistence of antibodies in Gambians. Possible reasons include genetic and environmental factors, depressed immunological reactivity associated with concurrent infection (notably parasitic diseases), and unusually high rates of synthesis and catabolism of immunoglobulins. The value of transportable immunoplates for serological surveys and for accurate assessment of antibody persistence is discussed.

In many areas of the world, the epidemiology of influenza is recorded only sporadically or remains undocumented. Such an area is tropical West Africa. Two factors have recently made possible a retrospective, longitudinal, seroepidemiological survey of influenza A infection in the Gambia over the period 1967–1974. First, the development of the single radial diffusion (SRD) technique (*1*, *2*, *3*) for the assay of antibodies to the surface antigens (haemagglutinin and neuraminidase) of influenza viruses has made possible rapid and precise antibody assays on small volumes of serum using immunoplates that can be sent by mail to laboratories where they are required. Second, because of continued monitoring of malaria in the Gambia by the British Medical Research Council, sequential serum samples from known donors were available that had been collected during investigations made in several rural Gambian villages.

The present paper describes the use of the SRD technique to detect and assay antibodies against A/Hong Kong/68 (H3N2) influenza virus in sera from residents of two Gambian villages during periods when epidemics of influenza-like illness were known to be occurring.

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MATERIALS AND METHODS

Human sera

The sera examined were collected from residents of two Gambian villages, Manduar and Kafuta, and stored at −20°C until used. (For a description of the environment in rural Gambia, see McGregor (*4*).) The samples from Manduar relate to 67 donors and were taken by venipuncture on 13 occasions at intervals of several months during 1967–1974. Those from Kafuta were obtained by finger prick from 27 individuals at approximately monthly intervals over the period May 1968–May 1969. These latter individuals were, at the time of the study, receiving Dapolar (cycloguanil embonate plus acedapsone), either alone or in combination with amodiaquine, as protection against malaria (*5*).

The collection of sequential serum samples from schoolboys in the United Kingdom who were 11–12 years of age in 1970 has been described previously (*6*). A small number of sequential sera from adults infected with A/Hong Kong/68 virus in 1969–1970 were made available by epidemiological laboratories in the United Kingdom.

Virus strains and specific antisera

The X31 high growth yield recombinant strain antigenically identical to A/Hong Kong/68 (H3N2) [a] virus (*8*) and the recombinant X15 HK strains of antigenic composition A/equine/Prague/1/56 (Heq1)–Hong Kong/68 (N2) and A/Hong Kong/68 (H3)–A/equine/Prague/56 (Neq1) were received from Dr E. D. Kilbourne, Mount Sinai School of Medicine, New York, USA. These antigens are referred to as H3N2, Heq1N2, and H3Neq1, respectively. A/chicken/Germany/'N'/49 (Hav2Neq1) was from the collection of the WHO Collaborating Centre for Influenza, London, England.

Antisera to the purified haemagglutinin (HA) and neuraminidase (NA) antigens of the A/Hong Kong/68 (H3N2) virus were as previously described (*1*). Antisera to influenza A nucleoprotein (NP) and matrix protein (MP) were as described by Schild (*9*).

SRD tests

Tests were performed with immunoplates [b] composed of agarose gels containing influenza antigens at standard concentrations of 0.2 mg virus protein per ml of gel as described previously (*1, 3, 10, 11, 12*). Most studies were carried out with immunoplates containing intact H3N2 virus. Such plates detect antibody to the two surface antigens of the influenza virus particle, the HA antigen (H3) and the NA antigen (N2) of A/Hong Kong/68. In order to characterize the antibodies further, selected sera were tested on plates containing recombinant influenza viruses. Plates containing intact Heq1N2 recombinant virus were used to detect antibody to NA (N2), whilst plates containing the " reverse " recombinant H3Neq1 were used to detect antibody to HA (H3). Antibodies to the internal antigens of influenza A virus (NP and MP) were assayed as described previously (*3, 10, 12*) using immunoplates containing a detergent-disrupted A/chicken/Germany/'N'/49 (Hav2Neq1) virus, which possesses surface antigens distinct from those of human influenza virus.

Immunoplates were sent to the Gambia by air freight for the addition of test sera and the results of the antibody assays were read from plates returned to London after 1–3 weeks. Readings were made without knowledge of the identity of the serum donors. The plates were transported in moist airtight containers without refrigeration. Spoilage occurred in only one of the seven batches sent to the Gambia; the satisfactory condition of the remaining plates was confirmed by the reactions of the reference antisera. Immunoplates each containing 56 2-mm-diameter wells were employed. Five-μl volumes of each test serum were introduced into the wells and the diameter of zones of opalescence developing around antibody-containing wells were measured with a micrometer eyepiece scale (*10*). Reference antisera to A/Hong Kong/68 haemagglutinin and neuraminidase were applied to each plate as controls. In addition, appropriate antisera were included in all plates used to assay antibodies to the internal antigens (NP and MP) of the influenza virus. Antibody titres were expressed as zone annulus areas (mm^2) per 5 μl of serum. For purposes of analysis, an increase of 35% in the area of opalescence in the SRD test between paired serum samples was considered to be evidence of a significant antibody rise for all virus antigens used in the tests (*13*).

RESULTS

Manduar sera

The typical appearance of an immunoplate containing intact A/Hong Kong/68 virus antigen, on which were tested sequential serum samples col-

[a] For the nomenclature of influenza virus antigens, see ref. 7.

[b] Hyland Laboratories, Costa Mesa, CA, USA.

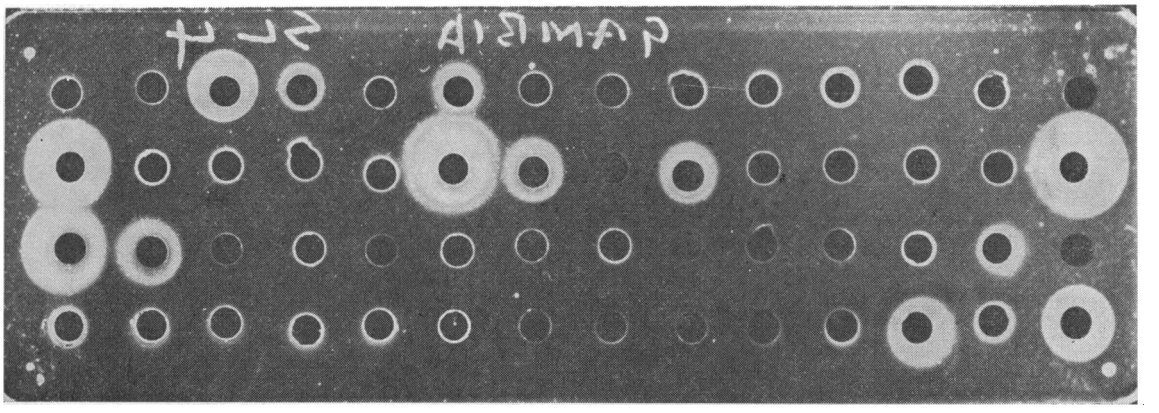

Fig. 1. An SRD immunoplate containing intact A/Hong Kong/68 virus (X31 strain) showing zones of opalescence around sera containing antibodies to the surface antigens of the A/Hong Kong/68 virus. The annulus areas of the zones of opalescence are directly proportional to the concentration of antibody in each test serum. The wells contain representative sequential serum samples from Manduar residents and are numbered from left to right and from top to bottom of the figure. Wells 1–4 contain sera collected in November 1970, March 1971, November 1971, and February 1972 from subject M154. Wells 5–12 contain sera collected in March 1968, March 1969, November 1969, March 1970, November 1970, March 1971, November 1971, and February 1972 from subject M177. Wells 13 and 15–21 contain sera collected on the same dates from subject M180 and wells 22–27, 29, and 30 contain sera from subject M198. It is seen that M154 was infected with A/Hong Kong/68-like virus between March and November 1971. M177 was infected between March 1968 and March 1969 and reinfected in 1971. Similarly, M180 and M198 were infected in 1968–1969 and reinfected in 1971. Wells 28 and 31 contain reference rabbit immune antisera to Hong Kong/68 neuraminidase and haemagglutinin, respectively.

lected between 1968 and 1972 from several residents of Manduar village, is shown in Fig. 1. The acquisition of antibody to the surface antigens (HA and NA) of the test virus, and also changes in antibody levels as indicated by the sizes of the zones of opalescence, can readily be seen.

The results of antibody assays made on sequential serum samples from representative individual donors are shown in Table 1; for the group as a whole, the incidence of significant antibody rises between consecutive serum samples for the period 1967–1974 is summarized in Table 2. Fig. 2 shows the prevalence and mean levels, expressed as the mean annulus areas of positive sera, of antibodies in Manduar residents from 1967 to 1974. In these studies employing H3N2 antigen, antibody to both the HA (H3) and the NA (N2) antigens of the test virus contributed to the development of zones of opalescence and the specificity of the antibody responses was not determined routinely. However, additional tests described below were performed to identify the specificity of some antibody responses.

The data from Manduar permit one to make several observations. Apart from four samples taken in March 1968, in which antibodies were found in low concentrations (range 0.7–1.8 mm²), sera taken in the first year of the study contained no antibodies. It is probable that the positive sera contained antibody to

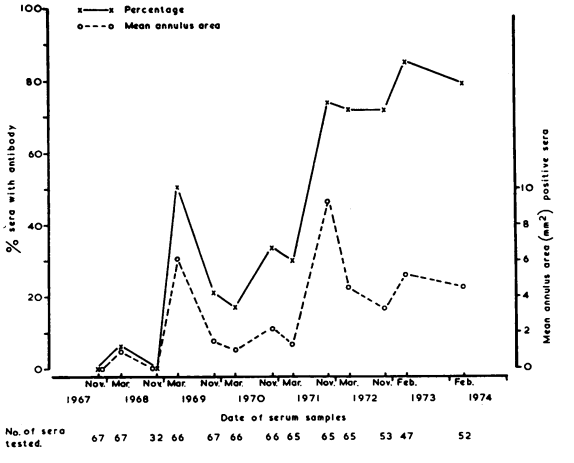

Fig. 2. The prevalence of influenza antibodies in sera collected from Manduar residents between 1967 and 1974 and the mean antibody titre (mean annulus area in mm²) of positive samples.

Table 1. Antibody concentrations[a] (annulus areas in mm²) in sera from representative individual residents of Manduar

Donor	Age at Nov. 1967 (years)	Sex	Sampling date											
			Nov. 1967	Mar. 1968	Nov. 1968	Mar. 1969	Nov. 1969	Mar. 1970	Nov. 1970	Mar. 1971	Nov. 1971	Mar. 1972	Nov. 1972	Feb. 1973	Feb. 1974
M 180	4	F	0	0	0	25.1	1.0	0.7	0	0	34.2	10.7	6.5	4.9	5.4
M 171	5	F	0	0	0	10.1	0	0	1.0	1.0	30.0	12.8	11.4	11.4	13.5
M 123	6	F	0	0	NS[b]	18.9	2.2	1.1	9.4	1.0	1.0	0.7	1.8	8.2	3.9
M 154	7	M	0	0	NS	12.1	0.7	0.7	0.7	0.7	13.5	3.9	3.9	3.9	3.9
M 339	15	F	0	0	0	2.6	0	0	1.0	1.0	2.6	1.8	1.4	10.1	2.2
M 92	19	M	0	0	0	1.8	0	0	0	0	2.2	1.4	1.0	NS	2.6
M 49	25	M	0	0	0	0	0	0	0	0	10.1	6.5	3.9	2.6	4.4
M 96	44	M	0	1.8	NS	1.8	1.0	0.7	0	0	3.0	1.8	0.7	9.4	1.4
M 75	46	M	0	0	NS	2.6	1.0	0.7	0	0	10.1	6.5	1.8	1.8	1.8
M 352	49	F	0	0	NS	2.6	0	0	0	0	1.8	1.8	1.8	3.0	1.8

[a] Tests made with A/Hong Kong/68 virus.
[b] NS = no sample.

NA (N2) induced by infection with "Asian" (H2N2) virus before Hong Kong virus became common. The high prevalence and titre of antibodies recorded in sera taken in March 1969 (Fig. 2 and Table 2) therefore indicate that infection with the Hong Kong virus first became frequent among villagers in the period November 1968–March 1969. Findings imply that prior to peak intensity, i.e., in the months immediately preceding November 1971, transmission of the virus in Manduar

Table 2. The number and percentage of consecutively paired serum samples from the same Manduar donors showing increases of 35 % or greater in the concentration of antibody to A/Hong Kong/68 virus in SRD tests

Time between pairs of serum samples (months)	Antibody increase		Number of sera showing antibody increases of:		
	No.	%	35 %-99 %	100 %-199 %	200 + %
Nov. 1967–Mar. 1968 (4)	4/67	5.97	—	—	4
Mar. 1968–Nov. 1968 (8)	0/32	—	—	—	—
Nov. 1968–Mar. 1969 (4)	10/32	31.25	—	—	10
Mar. 1969–Nov. 1969 (8)	2/65	3.08	—	—	2
Nov. 1969–Mar. 1970 (4)	0/66	—	—	—	—
Mar. 1970–Nov. 1970 (8)	15/65	23.08	—	—	15
Nov. 1970–Mar. 1971 (4)	0/65	—	—	—	—
Mar. 1971–Nov. 1971 (8)	39/65	60.00	—	1	38
Nov. 1971–Mar. 1972 (4)	2/65	3.08	1	—	1
Mar. 1972–Nov. 1972 (8)	8/53	15.09	3	2	3
Nov. 1972–Feb. 1973 (3)	17/41	41.46	2	1	14
Feb. 1973–Feb. 1974 (12)	7/44	15.91	7	—	—

occurred in discrete episodes separated by intervals in which no evidence of transmission was discernible. Subsequently, however, transmission appeared to persist continuously, varying in intensity but becoming particularly frequent again between November 1972 and February 1973.

Following the first widespread epidemic in March 1969, antibody concentrations in sera were poorly maintained (Fig. 2 and Table 1). By November 1969, antibodies were no longer detectable in 21 out of 33 individuals whose sera had contained them 8 months earlier. The rate of fall was not related to the initial concentration of antibody or to the age or sex of the donors. After reinfection, individual antibody levels were better maintained. For the first 4 years of the study period, increases in antibody in sera from individual donors were clearly discernible since virtually all appeared as increases in annulus area of more than 200%. After November 1971, however, smaller increases predominated except in the period November 1972–February 1973 (Table 2).

In the group as a whole, the frequency of infection was assessed by interpreting an increase in antibody concentration of 35% or more in consecutive serum samples as evidence of new infection. Five individuals appeared to escape infection; this conclusion is based on the complete absence of antibody in serum samples taken from 1 person on all 13 occasions, from 2 persons on each of 11 occasions, and from 2 persons on each of 6 occasions. All the remaining 62 persons became infected; 16 were infected once, 32 twice, 13 three times, and 1 four times during the study period.

Table 3 shows the specificity of the antibody elicited during the increases in antibody in a number of Manduar residents during the first and second major waves of infection, i.e., in 1968–1969 and 1971. SRD tests were carried out with immunoplates containing H3N2 virus and the H3Neq1 and Heq1N2 recombinants to detect antibody specific to the HA and NA antigens of A/Hong Kong/68 virus and with immunoplates containing detergent-disrupted A/chicken/Germany/'N'/49 virus to detect antibody to the internal antigens of influenza A virus, i.e., NP and MP. Nineteen individuals who were infected between November 1968 and March 1969 were fully tested. During the first wave of infection, only 7 of the 19 showed increases in antibody to both the HA and NA of A/Hong Kong/68 and the remaining 12 developed antibody to NA only. All 19 of those who showed increases in antibody to one or both of the surface antigens also developed antibody to NP but no increase in antibody to MP was detected. Twelve of the 19 individuals showed increases in antibody in one or more of the test systems between March and November 1971. However, the pattern was markedly different from that in 1968–1969: 10 of the 12 showed increased antibody to both HA and NA whereas only two individuals had increased antibody to only one of the two antigens. Eight of the 12 showed a rise in antibody to NP.

Kafuta sera

Sera from the 27 Kafuta residents were assayed on immunoplates using H3N2 antigen. In samples taken in May, June, July, August, September, October, and December 1968, evidence of antibody

Table 3. Specificity of antibody detected in the sera of 19 Manduar residents infected with A/Hong Kong/68 virus in the period November 1968–March 1969 and of 12 of the same individuals reinfected during March–November 1971

Dates of consecutive serum samples	Number (and percentage) of individuals showing an increase in antibody					
	virus surface antigens				virus internal antigens	
	H3N2 [a]	H3+N2 [b c]	H3 only [b]	N2 only [c]	NP [d]	MP [d]
Nov. 1968–Mar. 1969	19 (100)	7 (36.8)	0 (0)	12 (63.1)	19 (100)	0 (0)
Mar.–Nov. 1971	12 (100)	10 (83.3)	1 (8.3)	1 (8.3)	8 (66.6)	0 (0)

[a] Immunoplates containing X31 virus; antibody increases detected but not identified as specific antibody to haemagglutinin, neuraminidase, or both.

[b] Antibody to H3 detected using immunoplates containing H3Neq1 recombinant.

[c] Antibody to N2 detected using immunoplates containing Heq1N2 recombinant.

[d] Antibody detected using immunoplates containing detergent-disrupted A/chicken/Germany/'N'/49 (Hav2Neq1) virus.

Table 4. Antibody concentrations[a] (annulus area in mm²) in sequential serum samples from 6 residents of Kafuta who became infected with influenza in the period Jan.–March 1969

Donor	Age (years)	Sex	Date of sample				
			7 Dec. 1968	6 Jan. 1969	7 Mar. 1969	7 Apr. 1969	7 May 1969
K.24	5	M	0	0	8.2	4.9	1.8
K.25	6	M	0	0	4.9	2.2	1.0
K.28	6	F	0	3.0	10.1	3.9	0
K.44	18	M	0	0	4.9	2.6	0.7
K.122	5	M	0	0	10.1	4.4	1.8
K.165	18	F	0	0	12.8	6.5	3.9

[a] Tests made with A/Hong Kong/68 virus.

was found in only one instance, the September sample obtained from a 3-year-old child. The antibody titre was low (1.0 mm²) and the serum of this child remained negative throughout the rest of the investigation. In January 1969, antibody appeared in the serum of another individual and in March 1969 in sera from a further five donors. The findings relating to these last six individuals from December 1968 onwards are summarized in Table 4.

These results imply that in Kafuta village, extensive transmission of the Hong Kong influenza virus first occurred in January and February 1969. Table 4 illustrates clearly how rapidly antibody levels decayed after reaching peak concentrations. The half-life of antibody in the six infected individuals was 20–42 days with a mean of 28 days. The half-lives of the antibody in the two teenage members of the group (Table 3) were 25 and 37 days; these fell within the range detected for the remainder, who were children aged 5–6 years.

Persistence of antibody in Caucasian populations

Table 5 shows the results of SRD tests, using immunoplates containing A/Hong Kong/68 virus, on a representative selection of sequential sera from United Kingdom adults and schoolboys infected with A/Hong Kong/68 or A/England/42/72 virus at various times from 1969 to 1972. The SRD tests on the schoolboys' sera were carried out in a similar manner to those on the Gambian sera: immunoplates were sent by post to the Public Health Laboratory, Guildford[a] for the addition of sera and returned to London for the reading of the results. Persistence of antibody for the duration of the study (1–3 years) was evident in sera from both the adults and the children. In none of the 10 adults or 20 children tested was the antibody level following infection reduced to undetectable concentrations during the period of study. The time intervals between serum samples were too large for the accurate calculation of antibody half-life. However, for both the adults and the children the mean half-life was greater than 1 year, compared with 28 days for the Kafuta subjects.

DISCUSSION

Chakraverty et al. (*2*) and Mostow et al. (*13*) considered that the SRD test was a convenient and reliable technique for antibody assays with influenza viruses and was well suited to seroepidemiological studies. Our experience in the present studies, employing immunoplates transported from the United Kingdom to the Gambia, agrees with this view and the studies described here provide new information on the occurrence of influenza in a tropical African community.

The findings described above indicate that, in the villages of Manduar and Kafuta, extensive transmission of A/Hong Kong/68 influenza virus occurred for the first time between December 1968 and March 1969 and that in Manduar, further epidemic episodes involving the same virus or antigenically closely related strains occurred at irregular intervals, the most extensive being just prior to November 1971.

Comparison of findings in the Gambia with events in other areas is of interest. Although relatively little is known of its pattern of spread in Africa, the epidemiological behaviour of A/Hong Kong/68 (H3N2) virus is better documented than any previous pandemic influenza virus. Its first isolation in July 1968, its antigenic characteristics, and its early spread are described elsewhere (*14*, *15*, *16*). Severe outbreaks associated with the Hong Kong virus occurred in the USA in the first season of its prevalence (late 1968 to early 1969), whereas in most other areas of the world the epidemiological impact of the virus during this period was small. In contrast,

[a] SRD tests were carried out by Mr A. J. Smith of Guildford Public Health Laboratory, England, on serum samples from schoolboys with a known history of influenza infection (*6*).

Table 5. Antibody concentration to A/Hong Kong/68 virus (annulus area in mm²) in sequential serum samples from residents of the United Kingdom infected with A/Hong Kong/68 or A/England/42/72 viruses

Donor	Age (years)	Sex	Date of serum sample								
			Oct.-Dec. 1969	Jan.-Feb. 1970	Oct.-Dec. 1970	Oct.-Dec. 1971	Oct.-Nov. 1972	Jan. 1973	Oct.-Nov. 1973	Apr.-May 1974	Nov. 1974
157 [a]	52	F	0	12.5	9.7	7.1	—	—	—	—	—
197 [a]	32	M	0	5.6	3.9	2.5	—	—	—	—	—
230 [a]	39	M	0.3	19.7	12.1	4.9	4.9	—	—	—	—
294 [a]	57	M	0	4.9	2.2	1.8	—	—	—	—	—
299 [a]	65	M	0	15.7	11.4	6.7	—	—	—	—	—
67 [b]	11–12	M	—	—	—	—	0.5	7.1	4.9	3.5	—
139 [b]	11–12	M	—	—	—	—	0	3.0	1.8	1.8	1.8
170 [b]	11–12	M	—	—	—	—	0	8.2	8.2	—	—
930 [b]	11–12	M	—	—	—	—	0	9.4	5.9	—	—
259 [b]	11–12	M	—	—	—	—	0	7.6	3.9	2.6	—
497 [b]	11–12	M	—	—	—	—	0	9.4	8.2	6.5	6.5
753 [b]	11–12	M	—	—	—	—	0.7	7.0	3.0	2.2	—
961 [b]	11–12	M	—	—	—	—	0	10.1	8.2	4.9	—
2008 [b]	11–12	M	—	—	—	—	0	6.5	—	3.9	—
2014 [b]	11–12	M	—	—	—	—	0.7	5.4	3.9	—	—
2078 [b]	11–12	M	—	—	—	—	0	4.9	3.0	—	—
2125 [b]	11–12	M	—	—	—	—	0	30.4	16.5	—	—
29 [a]	11–12	M	—	—	—	0	7.0	—	6.5	9.4 [c]	—
239 [a]	11–12	M	—	—	—	0	1.8	—	1.4	—	—
257 [a]	11–12	M	—	—	—	0	7.6	—	5.9	7.0 [c]	—
276 [a]	11–12	M	—	—	—	0	3.9	—	3.0	3.9 [c]	—
378 [a]	11–12	M	—	—	—	0	12.8	—	11.4	11.4	—
502 [a]	11–12	M	—	—	—	0	4.9	—	3.9	3.0	—

[a] A/Hong Kong/68 infections.
[b] A/England/42/72 infections.
[c] Subjects probably reinfected with A/Port Chalmers/73 virus.

the same virus produced severe outbreaks in many areas during the same season a year later but had only a minor impact in the USA (*17*). Despite the appearance of an antigenic variant (A/Hong Kong/5/72), which had a restricted international distribution in 1971, the A/Hong Kong/68 virus remained the most common influenza A virus until late 1972–early 1973, when it was replaced by the A/England/42/72 (H3N2) virus containing variant HA and NA surface antigens (*18*). Subsequently, the variant A/Port Chalmers/1/73 (H3N2), which showed evidence of further antigenic " drift " from A/Hong Kong/68 virus (*19*), became common during late 1973 and early 1974 and persisted during the following year.

The isolation of influenza viruses in the Gambia has not been attempted. However, World Health Organization reports (*20*) document the limited available information on isolation in Africa. These reports indicate that the prevalence of influenza A virus strains in tropical and North Africa reflects that of the northern hemisphere in general and of

Europe in particular, whereas in southern Africa changes in the prevalence of successive variants occur later than in the northern hemisphere. In the period 1967–early 1968, influenza activity in Europe and southern Africa was associated with the "Asian" virus but comparable information was not reported from other regions of Africa. Between December 1968 and September 1969, isolates of A/Hong Kong/68 virus were made in tropical and North Africa (Kenya, Senegal, and Egypt) but the epidemiological impact of influenza was generally low. A few isolations of A/Hong Kong/68 were made in the same areas between October 1969 and April 1970 and in this period influenza activity was also generally low, although a country neighbouring the Gambia, Senegal, reported a high incidence of infection. From February to July 1971, outbreaks of moderate to high severity were reported from the Canary Islands, Senegal, and South Africa. During late 1971 and early 1972, A/Hong Kong/68 remained the prevalent variant but in the same season a year later, moderate to high levels of influenza in tropical and North Africa were associated with the A/England/42/72 variant. A further year later, in 1973–1974, A/Port Chalmers/73 virus was mildly active in these areas and was detected in Senegal.

For Manduar, data on the prevalence and titre of antibodies (Fig. 2) and on the detection of significant rises in antibody concentration in paired sera indicate the same times of virus transmission except for the periods March–November 1972 and February 1973–February 1974. In these two periods the increased antibody levels recorded, although they fulfilled the criterion suggested by Mostow et al. (*13*) (i.e., $\geqslant 35\%$), were frequently low compared with increases observed in association with earlier epidemics. The significance of these relatively small increases is uncertain; while they may not be truly indicative of reinfection, it is more likely that they represent reinfection with antigenic variants of the Hong Kong/68 virus. In support of this view is the fact that the SRD test is broadly reactive and will detect antibodies directed against antigenic determinants common for the haemagglutinin antigens of influenza viruses of the H3 subtype that were prevalent between 1968 and 1975 (*12*). Further, unpublished observations (G. C. Schild, 1977) have shown that the SRD test employing immunoplates containing A/Hong Kong/68 virus as antigen will detect antibodies to A/England/42/72 and A/Port Chalmers/73 efficiently but that antibody levels (zone annulus areas) are smaller than those noted following the use of homologous antigen. Further support comes from the isolation in Senegal of the A/England/42/72 variant in 1972–1973 and of the A/Port Chalmers/73 variant in 1973–1974.

The conventionally used haemagglutination inhibition (HI) test is not well suited to the measurement of changes in the titre of antibody to influenza in longitudinal studies of individuals using sequential serum samples. To be significant, variations in HI titre must be at least two- to four-fold, i.e., 200–400% (*3*). In contrast, the relative precision afforded by the SRD test (*10*) makes it particularly valuable for surveys involving the measurement of fluctuations in antibody levels in serial serum samples, such as in the present study. For example, although the data from SRD tests (Table 5) show clear evidence of a slow but progressive decrease in antibody levels in children over a period of 1–3 years after infection, HI tests with the same sera failed to show significant decreases in antibody levels (*6*). The volumes of antisera available from Manduar and Kafuta were insufficient to enable conventional HI assays to be performed.

The different specificities of the antibody responses following primary infection of Manduar residents in late 1968 and 1969 and on reinfection in 1971 (Table 3) are worthy of comment. Only one-third of the population developed antibody to both HA and NA during their first infection with A/Hong Kong/68 virus whilst the remaining two-thirds developed antibody to NA only. On their second infection with H3N2 virus, 83% of individuals developed antibodies to both HA and NA. Similar observations on the failure to elicit an antibody response to HA on initial infection have not been recorded elsewhere and our observations from the Gambia may indicate a relative inability of Gambians to produce an antibody response on initial stimulus with a novel antigen, such as Hong Kong/68 HA (H3). The NA antigen (N2) of the A/Hong Kong/68 virus was not novel in 1968; related N2 antigens were present on influenza viruses of the "Asian" (H2N2) subtype that had been circulating since 1957. The development of antibody to the internal, type-specific NP antigen in a high proportion of Gambians showing a rise in the level of antibody to HA or NA is further confirmation of the nature of the infective agent as influenza A virus. No antibody to MP, the other internal antigen of the influenza virus, was detected. The significance of this antibody is not clear; Mostow et al. (*13*) detected its occurrence in sera from some 50% of individuals with clinically severe A/Hong Kong/68 infections but in only a small

proportion of individuals with mild or subclinical infections.

A noteworthy feature was the rapid decline in antibody prevalence and titre that occurred in both Manduar and Kafuta following the first epidemic. In the closely spaced studies made in Kafuta (Table 4), antibody concentrations in individual donors fell to low or undetectable levels within 3 months of acquisition. The serological studies made in Caucasian populations reported in this paper showed that, in such communities, levels of specific antibodies were well maintained for more than a year after infection with influenza A viruses. The results of only a few other studies on the persistence of influenza antibodies are available and no previous study has permitted estimates of antibody half-life to be made from closely spaced sequential serum samples as in the case of the Kafuta study reported here.

Smith & Davies (*6*) reported studies of the HI antibody titre to A/Hong Kong/68 virus or its variants in 11–12-year-old schoolboys in the United Kingdom. Following natural infection, HI antibody persisted for the duration of the study (2–4 years) with little change in level. Decreases in antibody levels were significant ($\geqslant$ 4 fold) in less than 10% of 56 subjects and all retained detectable antibody. Similar findings of antibody persistence were obtained in the same subjects for antibody to neuraminidase as detected by a modified HI test (*6*). For adults in the United Kingdom (M. S. Pereira, unpublished observations 1977), antibody to the Asian (H2N2) virus persisted well in sequential sera collected from 1957 to 1967 when examined by HI tests or neuraminidase inhibition (NI) tests (*21*). In contrast, Schild & Newman (*22*) investigated sera from a small number of individuals infected with Asian (H2N2) virus in 1957 and found that HI antibody was more persistent than NI antibody: whereas HI antibody could be detected 2 years after infection, NI antibody was reduced to undetectable levels within a year. The SRD tests performed on individuals in the United Kingdom (Table 5), including some of the subjects from the study of Smith & Davies (*6*), are more relevant for comparison with data from the Gambia. The long persistence of antibody detected by SRD in individuals from the United Kingdom is in striking contrast to the observations from the Gambia. In cases where the antibody specificity to HA, NA, and NP was estimated in Gambian sera, all antibodies were of short persistence.

Why antibody persistence in the Gambian communities should have been so poor is not obvious. In recent years, increasing evidence has indicated that the immune responsiveness of individuals to some bacterial and viral antigens can be adversely influenced by poor nutrition (*23*, *24*, *25*) and by concurrent infection, notably malaria (*26*, *27*). It is unlikely that such factors operated to an important extent in our Gambian communities since the antibody levels elicited following the primary infective episode were similar to those that occur in Caucasian sera. Moreover, the donors of the sera shown in Table 4 were known to have been free from malaria at the time of exposure to the influenza virus and to have been so for some months previously. Cohen & McGregor (*28*) reported daily rates of immunoglobulin (IgG) synthesis and catabolism in Gambians that were 6–7 times greater than those found in Caucasian communities, presumably the result of the frequent and heavy communicable disease challenge that the former sustain in their native environment. It is possible that unusually high immunoglobulin turnover rates may have in some way been responsible for the poor persistence of influenza antibody levels observed in the early part of this investigation. Further studies on this point seem indicated in view of the current emphasis on immunization procedures as a means of controlling communicable disease in developing countries. Knowledge of the classes of antibody contributing to the immune response to influenza in Gambians would also be important. Such investigations have not yet been attempted, although it has been established (G. C. Schild, unpublished observations, 1977) that the SRD test is capable of detecting both IgG and IgM antibodies to influenza.

Following the extensive virus transmission that occurred around November 1971, higher antibody concentrations were maintained throughout the remainder of the study period. It is probable that this represents better persistence of antibody after repeated infection with Hong Kong virus or its variants.

Reinfection with influenza appeared to occur frequently in many individuals. Unfortunately we have no evidence to allow comparison of the severity of clinical illness during the first and subsequent infections. We do know, however, that clinical manifestations of influenza among the general population of Manduar were no less obvious in the 1971 epidemic than in 1969. On a visit of 4 days' duration to the village in March 1969, 13 cases of influenza were diagnosed clinically whilst 18 cases were seen on a visit of the same duration made in November

1971. It may be, therefore, that immunity acquired by the population during the 1969 epidemic conferred little protection against infection sustained in a further major outbreak some 30 months later.

Further to the studies described in the present paper, an analysis of seroepidemiological surveys of influenza involving some 17 000 serum samples from 2000 individuals, the entire available population of four Gambian villages, is in progress and will be reported in due course.

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RÉSUMÉ

UTILISATION DE LAMES TRANSPORTABLES POUR IMMUNODIFFUSION RADIALE DANS DES ÉTUDES SÉRO-ÉPIDÉMIOLOGIQUES SUR LA GRIPPE EN GAMBIE

Des études séro-épidémiologiques sur la grippe ont été faites en Gambie au moyen de lames pour immunodiffusion radiale portant des antigènes grippaux, préparées d'avance et expédiées de Londres. La fréquence et la persistance des anticorps contre les antigènes de surface (hémagglutinine et neuraminidase) du virus A/Hong Kong/68 (H3N2), détectés par immunodiffusion radiale, ont été déterminées dans des séries d'échantillons de sérum prélevées de 1968 à 1974 sur des habitants sélectionnés de deux villages de Gambie, Manduar et Kafuta. L'emploi de lames transportables s'est révélé efficace pour la surveillance sérologique de la grippe et applicable aux études épidémiologiques dans les régions reculées ne disposant pas d'installations de laboratoire.

L'infection par le virus A/Hong Kong/68 (H3N2) ou ses proches variants antigéniques a été à plusieurs reprises largement répandue chez les habitants de Manduar entre 1968 et 1974. Trente pour cent de la population ont présenté des preuves sérologiques d'infection par le virus A/Hong Kong/68 entre novembre 1968 et mars 1969, et 60% entre mars et novembre 1971. Au cours de cette période, les mêmes individus ont été fréquemment réinfectés par le virus homologue ou un de ses variants antigéniques.

On a observé de façon frappante qu'en Gambie les anticorps sériques contre les antigènes (hémagglutinine et neuraminidase) du virus A/Hong Kong/68 ne persistaient que pendant un court laps de temps (demi-vie moyenne d'environ 28 jours), surtout après la première infection par un virus H3N2. Cette brève persistance de l'anticorps a été observée dans tous les groupes d'âge. Lors d'études comparables faites sur une population européenne (Royaume-Uni), chez laquelle les anticorps ont été mesurés par la même méthode, on a observé une bien plus longue persistance (demi-vie moyenne d'un an ou plus). Etant donné son haut degré de précision, de reproductibilité et d'aptitude à distinguer différentes teneurs en anticorps, l'immunodiffusion radiale constitue un excellent outil d'étude de la demi-vie des anticorps, contrairement à l'épreuve d'inhibition de l'hémagglutination qui ne distingue que médiocrement les différentes teneurs en anticorps et ne convient pas pour ce genre d'étude. La persistance relativement brève des anticorps chez les Gambiens pourrait être due à des facteurs génétiques et environnementaux, à une baisse de l'activité immunitaire peut-être associée à une infestation parasitaire, et à un rythme exceptionnellement élevé de synthèse et de catabolisme des immunoglobulines.

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