J. Hyg., Camb. (1978), **80**, 1
Printed in Great Britain

Single radial haemolysis: a survey of antibody titres in the Highland Region of Scotland to recent strains of Influenza A

By A. W. L. JOSS, J. K. McPHERSON and H. WILLIAMS
Microbiology Laboratories, Raigmore Hospital, Inverness

(*Received* 15 *March* 1977)

SUMMARY

The single radial haemolysis test is conveniently practical and economical and promises to have wide applicability in the study of influenza antibodies in human populations. It can also be adapted for preliminary examination of new virus isolates during epidemics.

Using this test a rather higher proportion of the population in the Highland Region of Scotland was found to possess antibody to a recent epidemic strain of influenza (A/Scotland/74) than was the case in the south of England. Antibody was detected and apparently evenly spread throughout all but the most remote island communities. Some evidence of the spread of the subsequent variant, A/Victoria/75, was obtained. Most of the school children in our study had high antibody titres to recent strains but the proportion with high antibody titres to these strains declined speedily from the age of 17 years onwards.

INTRODUCTION

Annual epidemics of influenza, centred on the winter months, are an expected feature of life in both the Northern and Southern Hemispheres. This is possible because of the ease with which the genetic material specifying influenza A surface antigens, notably the haemagglutinin (HA) and the neuraminidase (N), can mutate and recombine to produce variants able to escape the strictures of an increasingly immune population.

Major changes in these antigens, 'shifts', have produced such variants as A/Hong Kong/68 which first appeared in this region on the island of Lewis during the winter of 1969/70 (Buchan & Reid, 1972). Since 1969, only minor changes, 'drifts', have taken place, resulting in strains such as A/England/72 and A/Scotland/74.

A survey made in England and Wales in late 1975 (Pereira, 1975) indicated that few people possessed antibody to the more recent of these strains, A/Scotland/74. The A/Scotland/74 epidemic, however, began in north-east Scotland (Smith, 1975), and it was thought possible that a greater degree of immunity to this strain might exist in northern Scotland. We have therefore carried out a similar survey in the Highland Region, for comparative purposes, and at the same time evaluated the use of the single radial haemolysis (SRH) technique (Schild,

Pereira & Chakraverty, 1975; Russell, McCahon & Beare, 1975) in such population surveys.

MATERIALS AND METHODS

Virus strains

Influenza A/Victoria/3/75, A/Port Chalmers/1/73, A/England/42/72 and propiolactone-inactivated A/New Jersey/76 were kindly supplied by Dr J. J. Skehel, World Influenza Centre, London. The strain of influenza A/Scotland/74 was isolated in this laboratory. Virus was propagated in 10-day-old embryonated eggs, and harvested in the allantoic fluid. New virus isolations from throat swab specimens submitted to the laboratory were cultured in Rhesus monkey kidney cells.

Sera

Human sera were collected from hospital staff and geriatric patients, but the great majority were submitted to the laboratory for routine tests, mainly from antenatal women.

Ferret anti-A/Victoria/75 was supplied by Dr Skehel. Rabbit anti-A/England/72 was supplied by the Central Public Health Laboratory, Colindale, London. Human sera of known reactivity to A/England/72 were supplied by Dr G. C. Schild, National Institute for Biological Standards and Control, London.

Erythrocytes

Goose erythrocytes in Alsever's solution and human O erythrocytes in citrate phosphate dextrose were washed with phosphate-buffered saline pH 7·2 (PBS) three times before use.

Haemagglutination-inhibition assays

These were carried out as described by Pereira, Pereira & Law (1964), using human O erythrocytes.

Complement-fixation assays

The method recommended by Bradstreet & Taylor (1962) was used, with guinea-pig complement (Searle, High Wycombe, Bucks), horse haemolytic serum (Wellcome, Beckenham, Kent), sheep erythrocytes (Gibco Biocult, Glasgow) and anti-influenza serum and influenza antigen (The Standards Laboratory, Public Health Laboratory Service, London).

Single radial haemolysis

Our technique was a modification of that described by Schild *et al.* (1975). Virus was adsorbed to 10% goose erythrocytes (0·4 ml virus (HA 1024) per ml erythrocytes) at 4 °C for 10–15 min. The suspension was then washed twice in PBS and incorporated, to a final concentration of 1% erythrocytes, in 1·5% agarose (Indubiose A37, Micro-Bio Laboratories, London) containing PBS,

Table 1. *Comparison of SRH test with HI and CF tests*

Virus strain	Correlation with HI		Correlation with CF	
	Number of sera	Correlation coefficient	Number of sera	Correlation coefficient
A/England/72	54	0·69	29	0·59
A/Port Chalmers/73	35	0·64	–	–
A/Scotland/74	54	0·58	29	0·65
A/Victoria/75	–	–	29	0·68

0·1% sodium azide and 4 HC50 reconstituted guinea pig serum (Searle). The agarose, which had been maintained at 42–45 °C during the mixing procedure, was allowed to set in 10 cm Petri dishes (Sterilin, Teddington, Middlesex; 10 ml volume) or in 3 × 1 cm rectangular immunoplates (Hyland Travenol, Costa Mesa, California; 3 ml volume). Twenty-seven wells (2 mm diameter) were cut in the former; 8 in the latter. Control plates contained erythrocytes which had been sensitized with normal allantoic fluid. Four μl of serum, previously inactivated at 56 °C for 30 min, was added to each well. Diffusion and red cell lysis was allowed to proceed at 35 °C for 16 h, at which time the antibody titre was estimated as being proportional to the measured diameter of the zone of haemolysis around each well. Two control sera were included on each plate; one a negative control and the other yielding a zone of approximately 10 mm diameter. The titre of each serum on the plate was then adjusted to account for any deviation from the previously established mean values of the control sera.

RESULTS

The SRH technique

Our modification of the SRH technique was compared with that of the more conventional haemagglutination inhibition technique using a selection of sera which covered the range of titres experienced in the survey (Table 1). The correlation coefficients were similar to those obtained by Russell *et al.* (1975). The same degree of correlation was evident when the SRH test was compared with the complement fixation (CF) test, which is the standard serological technique for influenza A used in this laboratory (Table 1). On this occasion, the sera examined were those referred to the laboratory from suspected cases of influenza during the 1976 epidemic.

The SRH technique was very reproducible; the standard deviation in samples tested 58 times during a 3·5 month period was 5%, and equal to the error in reading zone diameters. We found it more reproducible than the HI technique.

Pereira (1975) defined three categories of serum HI antibody titre; high ($\geqslant$ 40), intermediate (10–20) and low ($<$ 10). We decided to divide our SRH titres into three approximately comparable categories. These were selected by comparing the two tests on doubling dilutions of a moderately high titre serum (Table 2). SRH titres of $>$ 8·0 mm were equivalent to high HI titres; 5·5–8·0 mm to inter-

Table 2. *Correlation of SRH and HI based on serial dilution of a single serum*

Serum dilution	SRH (mm)	HI	
1:1	10·0	240	High titre
1:2	9·0	120	
1:4	7·5	30	Intermediate titre
1:8	6·0	15	
1:16	5·5	10	
1:32	4·5	5	Low titre
1:64	3·5	< 5	
1:128	3·0	< 5	
1:256	2·5	< 5	
1:512	2	< 5	

Table 3. *Response of volunteers to vaccination with inactivated A/Scotland/74 + A/Port Chalmers/73 + B/Hong Kong/73. SRH titres against A/Scotland/74*

Vaccinees	SRH titre before vaccination	Number tested	Total	Number showing: No rise	Total	Rise: > 1 mm	Total	Number showing titres of: > 8 mm	> 11 mm
Old people	< 5 mm	28	41	8	13	20*	28	32	17
	⩾ 5 mm	13		5		8			
Staff	< 5 mm	7	15	0	0	7	15	15	9
	⩾ 5 mm	8		0		8			
Totals			56		13		43	47	26

* Including one case where titre increased from 3·5 to 7·5 mm.

mediate titres; and < 5·5 mm to low titres. Separate high and intermediate categories of SRH titre were desirable because it was noted that sera of known specificity, e.g. rabbit anti-A/England/72, or ferret anti-A/Victoria/75, when tested for cross-reaction with closely related heterologous virus strains, usually yielded zone diameters in the 7·0–8·0 mm range, but when tested against the homologous strain, the zone diameter was > 10 mm.

Response to vaccination with inactivated influenza

Sera were collected from a group of 56 volunteers (41 geriatric patients and 15 hospital staff) immediately before and approximately 3 weeks after vaccination with inactivated A/Scotland/74, A/Port Chalmers/73 and B/Hong Kong/73. The results are summarized in Table 3.

Of the 48 cases who were seropositive after vaccination, 47 had titres of > 8·0 mm. In more than half (26) of this group the titre was very high (> 11·0 mm). Twenty-one of the subjects tested possessed antibody to influenza before vaccination, but in 16 of these cases the antibody titre increased (by > 1 mm) after vaccination. Including the 27 people who sero-converted the total, therefore, in whom an increased titre was evident as a result of vaccination was 43 (77%). All 13 who showed no increased titre in response to vaccination belonged to the

https://doi.org/10.1017/S0022172400053341 Published online by Cambridge University Press

Table 4. *SRH titres to influenza strains in the Highland Region over the period November* 1975–*March* 1976

Virus strain in SRH test	Antibody titre (mm)	Nov.–Mar.	%	Nov.–Jan.	%	Feb.–Mar.	%
A/Scotland/74	< 5·5	548	36	326	42	222	29·5
	5·5–8·0	455	31	216	29	239	34
	> 8·0 (P)	22		6		16	
	> 8·0	500	33	225	29	275	36·5
A/England/72	< 5·5	497	32·5	302	39	195	26
	5·5–8·0	615	46·5	284	42	331	50·5
	> 8·0 (P)	90		42		48	
	> 8·0	323	21	145	19	178	23·5
A/Victoria/75	< 5·5	529	67	83	73·5	446	66·5
	5·5–8·0	197	28·5	25	26·5	172	28·5
	> 8·0 (P)	26		5		21	
	> 8·0	34	4·5	0	0	34	5

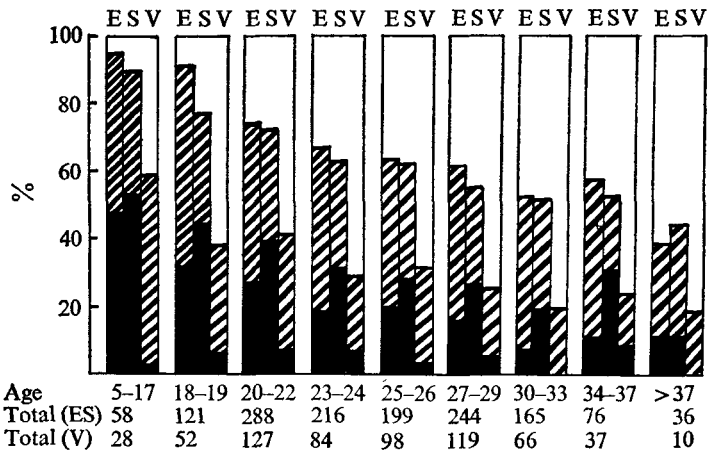
(P) = partial haemolysis, included in intermediate category.

geriatric group, 8 of whom were sero-negative before vaccination. The vaccine also contained influenza A/Port Chalmers/73 and antibody response to that strain was similar.

Antibody titres to influenza in the Highland Region of Scotland, November 1975–*March* 1976

A total of 1,525 serum specimens, the majority from antenatal patients, were examined between November 1975 and March 1976. The results are summarized in Table 4. About a third of the sample population lacked antibodies to any of the strains. Most of those in whom antibodies were detectable had higher titres against A/Scotland/74 than against A/England/72. After the outbreak in February 1976 of influenza due to the new strain A/Victoria/75, the percentage of sera which showed antibodies against A/Scotland/74 and A/England/72, as well as against the epidemic strain, increased.

Some sera collected before the epidemic apparently contained antibody to the epidemic strain. However, the collection date was two months before the outbreak, well before A/Victoria/75 was ever encountered within the region, so that all positive titres observed must have been the result of cross-reaction. The great majority of these cross-reactions gave rise to intermediate titres in the test, but a small number (4·5%) would have fallen into the high-titre category, if judged purely on the size of the zone of haemolysis (diameter > 8·0 mm). They could be distinguished from genuine high-titre sera on a qualitative basis, because in each instance it was observed that haemolysis was only partial. It would appear reasonable to consider this latter type of result as equivalent to an intermediate titre and, if the same principle is extended to the data concerning A/Scotland/74 and A/England/72 (Table 4), the principal effect is to reduce the proportion of sera with a high titre against A/England/72.



Age	5–17	18–19	20–22	23–24	25–26	27–29	30–33	34–37	>37
Total (ES)	58	121	288	216	199	244	165	76	36
Total (V)	28	52	127	84	98	119	66	37	10

Fig. 1. Relationship of influenza antibody level to age of donor. Total = number of specimens in group; E = antibody level to A/England/72; S = antibody level to A/Scotland/74; V = antibody level to A/Victoria/75. Percentage per group: high titre (> 8·0 mm), ■; intermediate titre (5·5–8·0 mm + > 8·0 mm, partial haemolysis), ▨; low titre (< 5·5 mm), □.

Age distribution

Because the population studied was largely antenatal patients the analysis is focused on the 18–38 age group. Fig. 1 shows that the proportion of individuals possessing antibody, especially high-titre antibody, to all three influenza strains diminishes in the older age groups. There is no preponderance of high-titre antibody in the younger groups to A/Victoria/75, as is the case with the other two strains.

Geographical distribution

We examined the data to see if there was any evidence to suggest the extent or even the route of spread of influenza in the region. The population is grouped in a semi-urbanized area around the Moray and Cromarty Firths, generally in the vicinity of Inverness itself, with small settlements in the more rural areas along the coast to the north and east. There are only two population centres of any size to the south, one a winter holiday area in the Spey valley, and the other around Fort William. The population in the extensive area to the west of the region consists of small, widely scattered communities, with a focus on Stornoway, a busy fishing port.

Antibody to A/Scotland/74 was found to be fairly evenly spread throughout these areas (Table 5). There are no real urban concentrations, but the populations of the largest towns, considered separately, possessed somewhat more than average antibody. In the rural population, there was a slightly higher proportion of sera with antibody to A/Scotland/74 in the inhabitants of the western area than in comparable groups living to the south and east of Inverness (Fig. 2). Only in the remote areas of the Northern and Southern Hebrides (2C, Table 5) did antibody to A/Scotland/74 drop to markedly lower proportions.

https://doi.org/10.1017/S0022172400053341 Published online by Cambridge University Press

Table 5. *Antibody to A/Scotland/74 and A/Victoria/75 in different areas of the Highland Region*

		A/Scotland/74			A/Victoria/75	
		Sample size	% Negative	% High titre	Sample size	% High titre
Total		1525	36	33	673	5
Town		557	33	35	235	6
Rural		920	38	31	416	5
Place of origin uncertain		48			22	
Inverness area	(1)	395	33	33	183	4
Inverness (34,800)*		237	31	34	114	6
Rural		158	37	31	69	2
West	(2)	298	37	34	145	6
Stornoway (5,200)		35	34	31	15	0
Rural		263	38	35	130	6
(2A)		32	13	72	19	10
(2B)		185	37	33	88	7
(2C)		46	59	15	23	0
North	(3)	380	37	32	159	3
Thurso (9,100) + Wick (7,600)		64	31	41	14	0
Rural		316	37	32	145	3
East	(4)	194	39	30	75	5
Nairn (8,200) + Forres (4,700)		120	38	38	51	8
Rural		74	41	16	24	0
Spey valley	(5)	63	43	38	30	13
South	(6)	147	34	28	59	5
Fort William (4,200) + Caol (3,700)		101	31	32	41	5
Rural		46	41	20	18	6

* Town populations in brackets.

The information on the spread of the more recent strain, A/Victoria/75, is relatively limited, because only the data from the later period of the survey are relevant. In fact, it is only the 5 % of cases with high titres who are likely to have encountered this strain, and when these were analysed on a regional basis (Table 5), the highest percentage was found in the Spey valley region. The same area produced an above average proportion of the isolations of influenza A achieved during the epidemic. Virtually all the remaining isolations came from the Inverness area or the west coast.

Strains were typed by comparing the zone sizes produced by reference sera on plates containing each strain as antigen with those on plates containing A/Victoria/75 and A/Scotland/74 respectively and they were all (14) found to be similar to A/Victoria/75. The similarity of the first strain to A/Victoria/75 was confirmed by HI (J. J. Skehel, personal communication). An example of the SRH typing is presented on Table VI and it shows that virus grown in tissue culture was as effective in the test as that from allantoic fluid.

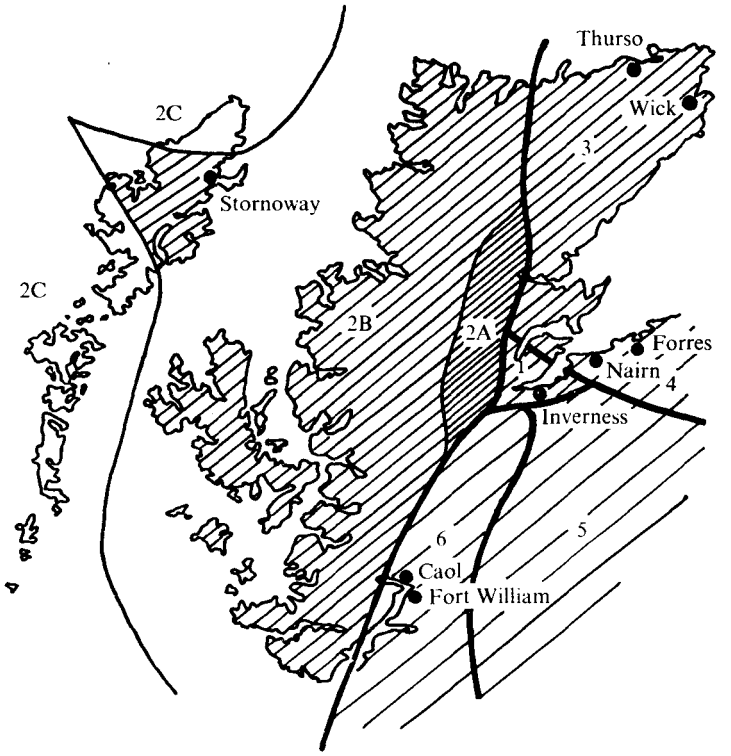


Fig. 2. Antibody to A/Scotland/74 – geographical distribution. Proportion of the population with high-titre antibody compared with the average for the region as a whole: greater than, ▨; equal to, ▨; less than, ▨; much less than, □.

Table 6. *Typing of isolates by the SRH technique*

Antiserum		Antigen				
		A/Scotland/74[a]	Isolate 1[a]	Isolate 2[b]	Isolate 3[b]	A/Victoria/75[a]
Rabbit anti-A/England/72		7·5	6·0	4·0	4·0	5·0 (P)
Human	Pre-vaccination[c]	6·0 (P)	2·0	2·0	2·0	2·0
	3 weeks post-vaccination	13·0	8·5 (P)	8·5 (P)	8·5 (P)	8·0
	3 months post-vaccination	12·0	7·5 (P)	7·0 (P)	7·0 (P)	7·0
Ferret anti-A/Victoria/75		8·5 (P)	14·0	13·0	13·5	13·0

[a] Virus in allantoic fluid.
[b] Virus from tissue culture, freeze thawed once.
[c] A/Scotland/74; A/Port Chalmers/73; B/Hong Kong/73 vaccine.
(P), partial haemolysis.

DISCUSSION

There is a need for a readily applicable test for population studies and surveillance of influenza, and the SRH test has advantages both in economy and reproducibility. If standard-sized Petri dishes are used, up to 27 tests may be performed under identical conditions on one plate, and when compared with the HI and CF tests, we found the correlation coefficients were similar to those of Russell *et al.* (1975).

https://doi.org/10.1017/S0022172400053341 Published online by Cambridge University Press

The zones ranged from 2 to 15 mm in diameter and the three categories chosen allowed comparison with the results of Pereira (1975) who, using the HI test, divided sera into those with titres of < 10, 10–20 and $\geqslant 40$ units respectively. Previous work (Moffet *et al.* 1964; Hobson *et al.* 1973), had indicated that titres of < 10 units gave no protection and > 40 afforded good protection against influenza. We found that when the antibody was known to be attributable to one particular strain, e.g. animal reference sera, or those arising from human seroconversion, the zones were always in excess of a diameter of 8·0 mm against the homologous strain, and generally less than 8·0 mm with strains which were not homologous.

The division of SRH results into these three categories was complicated by the occasional occurrence of partial haemolysis in some zones which were in excess of 8·0 mm diameter. The indications that such a result was due to cross-reaction were threefold: a few sera tested against A/Victoria/75 but collected two months before the strain appeared in the area; post-vaccination sera tested against heterologous strains; and heterologous reference sera tested against new isolates, all occasionally showed this type of reaction. Schild *et al.* (1975) also concluded that zones of partial haemolysis were due to cross-reaction. We recorded this type of result as intermediate, on the basis that by excluding them from the high-titre category we increased the probability that the latter resulted from prior exposure to the test strain. However, whether or not a high titre based on partial haemolysis is protective is uncertain.

The SRH test can be used to study changes in titre after vaccination. Seroconversion from an initially low titre to A/Scotland/74 was evident in 48 % of the group we studied, and if all increases in zone diameter by more than 1 mm are included more than three-quarters benefited by an increase in detectable antibody. SRH titres in the range 11·0–14·0 mm, attained by 46 % of those vaccinated, give an indication of the probable upper limit in recently exposed individuals, but titres of this order were not commonly experienced in the survey itself. All those in whom no response to vaccination was detected belonged to the geriatric group of patients.

In 52 % of the survey results, the titres to A/Scotland/74 and A/England/72 were identical (± 1 mm). The SRH test may be able to distinguish antibodies to closely related strains using antisera made under controlled conditions in the laboratory, but this is less easy in the more complex field situation. Conversely when new strains were incorporated in SRH plates and tested against reference sera, a presumptive identification of the A/Victoria/75 type was quite convincing. Moreover, this could be carried out using the contents of a single tissue-culture tube, thus avoiding passage in eggs.

Regarding the survey itself, the immediate conclusion is that fewer people in the Highland Region of Scotland, compared with England and Wales, were susceptible to A/Scotland/74. This might have been expected because, although A/Scotland/74 was first isolated in north-east Scotland (Smith, 1975), its appearance in this area was virtually simultaneous. There is some evidence that A/Scotland/74 made more impact in this area than the earlier strain, A/England/72,

for where exposure to both these drift-related strains had occurred, the antibody titre to the earlier strain should have been boosted (Fazekas de St Groth & Webster, 1966), but, since the titre to A/Scotland/74 was more often the higher, it seems that a greater proportion of the population had been exposed only to A/Scotland/74.

Because our data were collected mainly from antenatal women, the full age range was not covered, but it was clearly demonstrated that school children possessed the most antibody to all the strains tested, and that once an individual leaves school, the immune system increasingly loses touch with the constantly changing surface structure of the influenza virus. The high susceptibility of the oldest age group was confirmed in the data from the geriatric group before vaccination. Our results in some respects confirm what was previously known of influenza attack rates in different age groups (Carey *et al.* 1958; Dunn, Carey, Cohen & Martin, 1959; Chin *et al.* 1960; Hennessy *et al.* 1964; Buchan & Reid, 1972), even to the point of showing a small reversal – in the mid-30s age group – of the steady age-related decline in antibody supposedly brought about by the increased likelihood of contact with virus through having children at school (Chin *et al.* 1960; Buchan & Reid, 1972). Although the great majority of our youngest age group possessed antibody to recent strains, recent research (Smith & Davies, 1976) has shown that this was not necessarily the result of clinical influenza.

The survey contains data on two successive influenza strains, A/Scotland/74 and A/Victoria/75, and analysis on a geographical basis provides evidence on their spread in a widely scattered rural population. The greater proportion of high antibody titres to the previous winter's strain, A/Scotland/74, in the areas of highest population concentration, namely the eight largest towns, might have been predicted, but a surprising feature was the extent to which this strain had penetrated to all but the most remote areas of the west. Indeed, it appears to have spread more readily in the rest of the western area than in the rural populations to the south and east of the region. The first isolations of A/Scotland/74 in this region came from Gairloch, in the western area.

The succeeding strain, A/Victoria/75, was active in the south of Britain some time before the first isolation in this region was obtained from Grantown in the Spey valley. Influenza isolation represents only a small part of the evidence of an epidemic but, taken along with the serological data, it would seem that A/Victoria/75 was first introduced to the region from the south, in a winter holiday area. Subsequent isolations, however, from Inverness and the western area, together with the serological evidence, indicated a rapid spread in the region, especially in the central belt stretching through Inverness to the west.

Early entry of influenza strains to the remoter north-west has occurred before (Buchan & Reid, 1972), but the recent increased winter traffic due to winter sports and oil-related development may have facilitated the dissemination of influenza throughout this region in more recent years.

Experience in this survey shows that the SRH test has definite merit as a screening technique, and might be the way to screen for antibody to 'swine-type'

influenza, which at present is absent in the under-50 age group. The fact that propiolactone-inactivated A/New Jersey/76 can be used in the test, as we were able to demonstrate on a smaller number of pre- and post-vaccination sera, would avoid the hazards of disseminating live virus.

We are grateful to Mr A. Smith and Miss K. Shipp for statistical advice. We are also grateful to the staff of the routine virology and serology sections for access to new isolates of influenza A and the results of routine complement fixation tests.

REFERENCES

BRADSTREET, C. M. P. & TAYLOR, C. E. D. (1962). Technique of complement-fixation test applicable to the diagnosis of virus diseases. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **21**, 96–104.

BUCHAN, K. A. & REID, D. (1972). Epidemiological aspects of influenza in Lewis. *Health Bulletin* **30**, 183–6.

CAREY, D. E., DUNN, F. L., ROBINSON, R. Q., JENSEN, K. E. & MARTIN, J. D. (1958). Community-wide epidemic of Asian-strain influenza – clinical and subclinical illnesses among schoolchildren. *Journal of the American Medical Association* **167**, 1459–63.

CHIN, T. D. Y., FOLEY, J. F., DOTO, I. L., GRAVELLE, C. R. & WESTON, J. (1960). Morbidity and mortality characteristics of Asian strain influenza. *Public Health Reports (Washington)* **75**, 149–58.

DUNN, F. L., CAREY, D. E., COHEN, A. & MARTIN, J. D. (1959). Epidemiologic studies of Asian influenza in a Louisiana parish. *American Journal of Hygiene* **70**, 351–71.

FAZEKAS DE ST GROTH, S. & WEBSTER, R. G. (1966). Disquisitions on original antigenic sin. 1. Evidence in man. *Journal of Experimental Medicine* **124**, 331–45.

HENNESSY, A. V., DAVENPORT, F. M., HORTON, R. J. M., NAPIER, J. A. & FRANCIS, T., JR. (1964). Asian influenza: occurrence and recurrence, a community and family study. *Military Medicine* **129**, 38–50.

HOBSON, D., CURRY, R. L., BEARE, A. S. & WARD-GARDNER, A. (1973). The role of serum haemagglutination inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. *Journal of Hygiene* **70**, 767–77.

MOFFET, H. L., CRAMBLETT, H. G. & DOBBINS, J. (1964). Outbreak of influenza A2 among immunised children. *Journal of the American Medical Association* **190**, 806–10.

PEREIRA, H. G., PEREIRA, M. S. & LAW, V. G. (1964). Antigenic variants of influenza A2 virus. *Bulletin of the World Health Organization* **31**, 129–32.

PEREIRA, M. S. (1975). Influenza A antibody levels in the UK – 1975. *Communicable Disease Report* 47/75.

RUSSELL, S. M., MCCAHON, D. & BEARE, A. S. (1975). A single radial haemolysis technique for the measurement of influenza antibody. *Journal of General Virology* **27**, 1–10.

SCHILD, G. C., PEREIRA, M. S. & CHAKRAVERTY, P. (1975). Single radial haemolysis: a new method for the assay of antibody to influenza haemagglutinin. *Bulletin of the World Health Organization* **52**, 43–50.

SMITH, A. J. & DAVIES, J. R. (1976). Natural infection with influenza A (H3N2). The development, persistence and effect of antibodies to the surface antigens. *Journal of Hygiene* **77**, 271–82.

SMITH, J. W. G. (1975). Influenza, 1974–75. *Communicable Disease Report* 5/75.