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The incidence of *Herpesvirus hominis* antibody in the population

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INTRODUCTION

For a number of years the results of Buddingh, Schrum, Lanier & Guidry (1953) in New Orleans, U.S.A. have been quoted in the textbooks as showing that 90 % of the population over the age of 15 have neutralizing antibody to *Herpesvirus hominis*. The most complete British survey, reported by Holzel, Feldman, Tobin & Harper (1953), using a complement-fixation technique, showed a similar high incidence of antibody (86 %) in the same age groups.

These findings have been supported by many other surveys, although it has become apparent that the socio-economic background of the individuals in such a survey has an important influence on the detected incidence of antibody (Zinsser & Tang, 1929; Andrewes & Carmichael, 1930; Weyer, 1932; Burnet & Williams, 1939; Anderson & Hamilton, 1949; Hayward, 1949; Buddingh *et al.* 1953; Holzel *et al.* 1953; Coetzee, 1955; Dascomb, Adair & Rogers, 1955; Halonen, 1955; MacCallum, 1959; Stern, Elek, Millar & Anderson, 1959; Yoshino *et al.* 1962; Taglieri & Tresca, 1964; Kibrick & Gooding, 1965; Rodrigues & Carvalho, 1965; Becker, 1966).

In view of the suggestion of Yoshino *et al.* (1962) that a change in age distribution of antibody had occurred in Japan and as a period of 12 years had elapsed since the Holzel *et al.* (1953) report, it was thought worth while to determine the present incidence of both neutralizing and complement-fixing antibody in two populations in this country.

MATERIALS AND METHODS

Virus

A wild strain of *Herpesvirus hominis* producing rounded, ballooning degeneration of the tissue cells was used for the preparation of both the neutralizing and complement-fixing antigen in Edinburgh. A similar wild strain was used in the neutralization tests in Oxford.

Neutralizing antigen

In Edinburgh the antigen for the neutralizing antibody assays was produced in RK 13 cells (obtained from Moredun Research Institute, Edinburgh) grown in

199 medium (Glaxo) with 10% calf serum. Monolayer cultures in Roux bottles were infected with a multiplicity of greater than one and the cells harvested when the cytopathic effect was complete. To harvest the antigen, the medium was discarded and the infected cells removed by glass beads in 5 ml. medium per Roux bottle. This cell suspension was treated at full power in an MSE ultrasonicator for 5 min. and the resulting clear fluid used as the antigen. Infectivity titrations were carried out according to the method of Peutherer & Smith (1966) in BHK 21 cells (Macpherson & Stoker, 1962). At Oxford primary amnion cells were infected with a dose of virus which produced a cytopathic effect in every cell in 36–48 hr. The supernatant fluid was removed and centrifuged at 3000 rev./min. for 20 min. and the supernatant from this was removed for use. The infectivity of this supernatant was determined by inoculation of 0·1 ml. amounts of ten-fold dilutions into tubes of primary amnion tissue cultures.

Complement-fixing antigen

This was prepared in Edinburgh as for the neutralizing antigen except that the infected cells were removed from the glass in 5 ml. distilled water per Roux bottle. Suspensions were ultrasonicated and then centrifuged at 32,600 ***g*** for 30 min. in a Spinco model L ultracentrifuge using the SW 39 or SW 25 head to remove particulate matter. The supernatant was used as the complement-fixing antigen. Different batches of antigen were standardized against a reference herpes antiserum before use. It was found with both the reference herpes antiserum and the Colindale standard complement-fixing antiserum that the optimal dilution for the detection of herpes antibody was 1/32. For the purposes of this paper, the most concentrated antigen employed (a 1/4 dilution) will be referred to as the CF (conc.) antigen and the optimal dilution of antigen as the CF (opt.) antigen.

At Oxford, the optimum dilution of antigen was used in complement-fixation tests. The antigens used were kindly supplied by the Standards Laboratory, Central Public Health Laboratory, Colindale, and by Dr C. A. Ross at Ruchill Hospital, Glasgow (Grist, Ross, Bell & Stott, 1966). Sera were tested at 1/4–1/16. Those with titres of less than 1/8 were tested for neutralizing antibody.

Sera

All the Edinburgh sera were collected in the year 1965. They came from the following sources:

(1) 131 fourth-year medical students (of these 52 were Scottish born and educated).

(2) 75 probationer nurses from the Royal Infirmary, Edinburgh. These young women were bled during their period of preliminary training before they had been on duty in the wards.

(3) 376 specimens of acute phase serum submitted for diagnostic purposes to the Virology Unit, Bacteriology Department, Edinburgh University. These sera came from people living in the south-east of Scotland.

(4) 159 sera submitted to the Virology Unit of the Congenital Abnormalities

Research Unit of Edinburgh University. These specimens were from women of 16–43 years of age attending ante-natal clinics.

(5) 123 acute phase sera from patients admitted to the City Hospital, Edinburgh, and to the Royal Hospital for Sick Children, Edinburgh. These sera had all been submitted for virological investigation at the Wellcome Laboratory, City Hospital.

The Edinburgh age–antibody survey was carried out on 710 people comprising groups (3), (4) and (5) above together with 52 Scottish-born and -educated medical students. The Oxford sera were collected over the period 1962–65 and were from ante-natal women (18), medical neurological patients (95) and British-born medical students (52).

All sera were inactivated at 56° C. for 30 min. after dilution.

Neutralizing antibody assay

In Edinburgh this was carried out according to the accelerated adsorption technique of Peutherer & Smith (1966) using BHK 21 cells. Initially all sera were tested at dilutions of 1/8–1/256 against 100 TCID 50 of the virus. To save time, this was later altered to a screening technique, the dilutions tested being determined by the previously obtained complement-fixing antibody titre. Thus sera which had a complement-fixing antibody titre of $< 1/8$ were screened at dilutions of 1/8 and 1/16 in the neutralization test, while sera which were positive by the complement-fixation test were screened at dilutions of 1/128 and 1/256. Any sera which did not give an end-point at these dilutions were tested subsequently with the full range of dilutions, viz. 1/8–1/256. In Oxford the neutralizing antibody was assayed by a plaque reduction test. This test was carried out on all sera which did not show complement-fixing antibody. A 1/4 dilution of the inactivated serum was mixed with 50–100 plaque-forming units of virus. After 45 min at room temperature, equal volumes were inoculated to human amnion tissue cultures in test tubes. Sera which almost completely inhibited (90 % reduction) plaque formation were considered as positive.

Complement-fixing antibody assay

The Oxford sera were tested in agglutination trays (WHO) (Andrews & McDonald 1955) using an optimum dose of either of two antigens, $2\frac{1}{2}$ HD 50 of Burroughs Wellcome complement and overnight fixation at 4° C. In Edinburgh, the sera were tested as above except that 2HD 50 complement was used and a full checkerboard titration carried out on each serum. Doubling dilutions of the inactivated sera up to 1/1024 were prepared in Wasserman tubes before transfer to WHO Perspex plates. Standardized antigen at dilutions of 1/4–1/128 was used. Controls of each dilution of serum and antigen were included in each plate along with the appropriate complement controls.

RESULTS

Sera from 131 fourth-year Edinburgh medical students (average age 22 years) and 75 student nurses (average age 19 years) were examined for the presence of complement-fixing and neutralizing antibodies to *Herpesvirus hominis*. The results in Table 1 show that, in Edinburgh, only 40 % of the medical students and 48 % of the nurses have any detectable antibody. In Oxford, where 52 British-born medical students were examined, only 36 % were found to have detectable antibody.

Table 1. *The incidence of neutralizing and complement-fixing antibodies in sera from medical students and nurses*

Subjects	No. examined	Both neutralizing and CF antibody	Neutralizing antibody only	CF antibody only	Percentage showing either antibody
Edinburgh					
Medical students	131	46	3	3	40
Nurses	75	30	3	3	48
Oxford					
Medical students	52	—	—	—	36

Table 2. *The incidence of neutralizing and complement-fixing antibodies in the various age groups of subjects examined*

		Neutralizing antibody		Complement-fixing antibody			
				CF (conc.) Ag.		CF (opt.) Ag.	
Age	Total	No. positive	% positive	No. positive	% positive	No. positive	% positive
0–2 months	11	11	100	9	82	6	55
3–5 months	12	9	75	4	33	2	17
6–11 months	16	3	19	5	31	2	13
1 yr.	22	8	37	9	41	5	23
2 yr.	22	6	27	7	32	5	23
3 yr.	20	7	35	8	40	5	25
4 yr.	11	4	37	3	37	3	27
5 yr.	21	10	48	10	48	9	43
6–9 yr.	29	16	55	14	48	13	45
10–14 yr.	57	20	35	18	32	12	21
15–19 yr.	75	52	69	52	69	39	52
20–24 yr.	127	83	65	82	65	67	53
25–29 yr.	68	51	75	53	78	49	72
30–39 yr.	82	69	84	68	83	56	68
40–49 yr.	43	38	88	37	86	31	72
50–59 yr.	36	26	72	29	81	22	60
60–69 yr.	29	28	97	27	93	18	62
70– yr.	29	28	97	27	93	19	66

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Neutralizing antibody

The results of the antibody studies on the 710 sera from the Edinburgh area are shown in Table 2 and Fig. 1. Here a high incidence of neutralizing antibody (75–100 %) is found in children under 6 months. The incidence then declines to

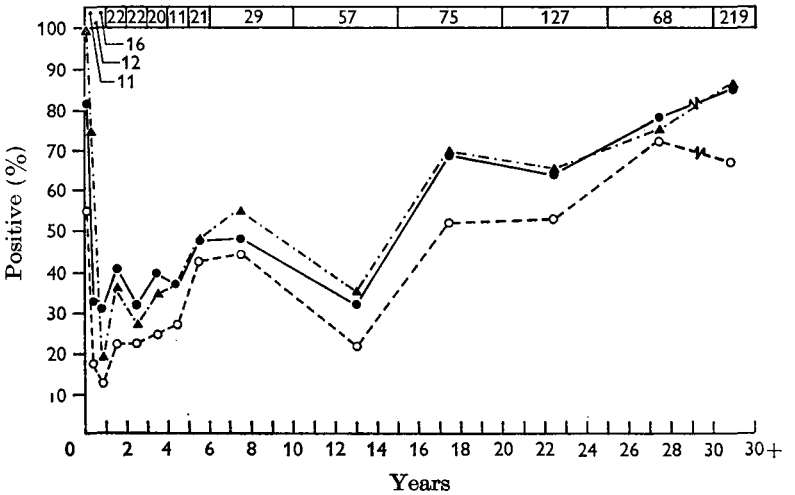

Fig. 1. The distribution of neutralizing and complement-fixing antibodies in the Edinburgh 1965 survey. ●——●, C.F. 1/4; ○— —○, C.F. 1/32; ▲—.—▲, neutralizing antibody.

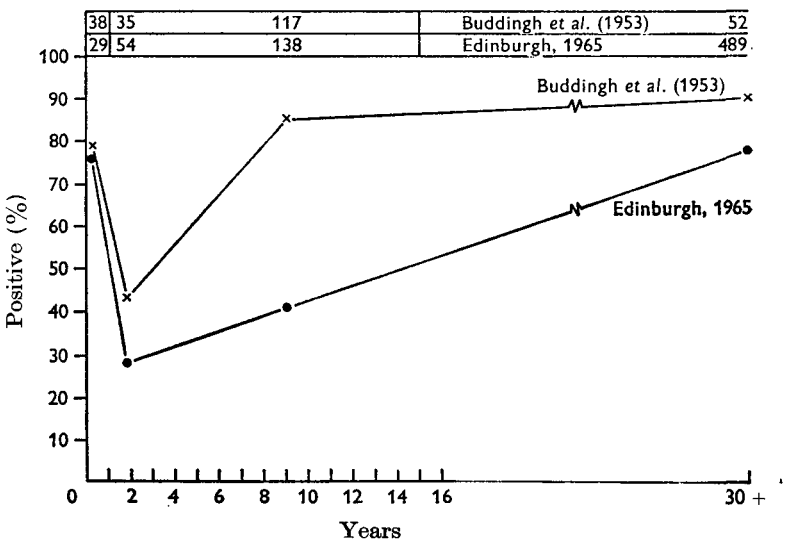

Fig. 2. Comparison of the incidence of neutralizing antibody in Edinburgh and New Orleans (Buddingh, Schrum, Lanier & Guidry, 1953).

19 % at 6–11 months, after which it rises slowly until 15–25 years when it is 65–69 %. Thereafter it increases to an ultimate 97 % in the over 60 age groups.

As the previous antibody surveys have all employed different age groupings, the

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results of this survey have been regrouped according to the methods of Buddingh *et al.* (1953) and Yoshino *et al.* (1962). These are expressed graphically in Figs. 2 and 3.

Comparison of the Edinburgh survey with the New Orleans results in white children of Buddingh *et al.* (1953) shows that in the under 6-month group, the incidence of antibody was very similar (76 and 79 %). In subsequent age groupings, however, a decreased incidence was recorded in Edinburgh despite the greater

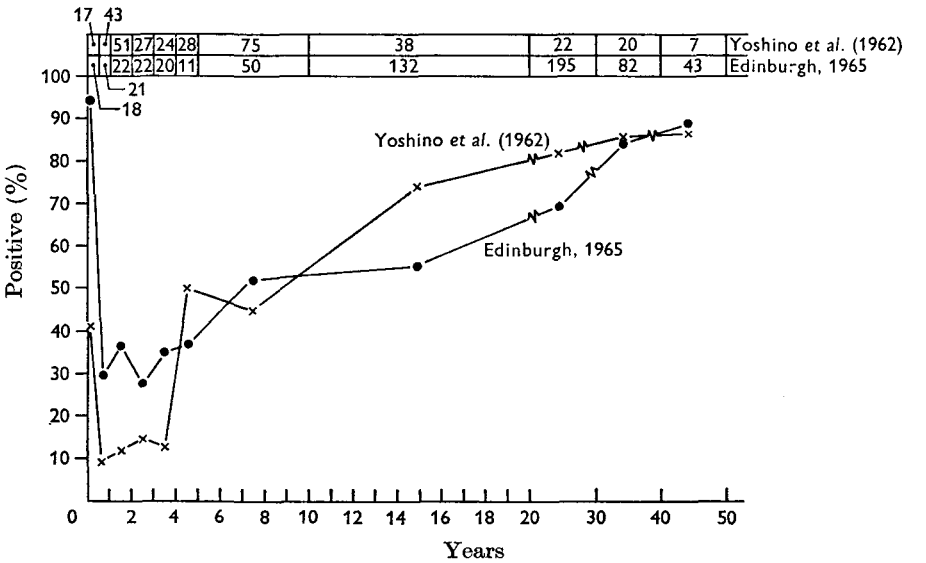

Fig. 3. Comparison of the incidence of neutralizing antibody in Edinburgh and Tokyo (Yoshino *et al.* 1962).

number of specimens examined. The decrease ranged from 15 % in the 7-month to 2-year group through 44 % at the 3–14 year group to 13 % in the over 14-year-olds. Figure 3 illustrates the comparison of the Edinburgh results with those of Yoshino *et al.* (1962) in Tokyo. Here it is noted that the results show a similar trend except for an increased incidence in Japan in the 10–19-year group. This may be a reflexion of the number of specimens examined in these age groups.

Complement-fixing antibody

The incidence of complement-fixing antibody in the Edinburgh sera is shown in Table 2 and Fig. 1, where the reaction to two different antigen concentrations is recorded. When the CF (conc.) antigen is used, the results obtained closely parallel those of the neutralization test except in the under 6-month group in which the incidence of neutralizing antibody is in excess of that of complement-fixing antibody. With the CF (opt.) antigen, the incidence of antibody is lower, especially in the younger and older age groups. In the 6-month–1-year and 1–2-year groups, the CF (opt.) antigen detected 18 % and 17 % fewer children with antibody compared with the CF (conc.) antigen while in the over-30-year age group the decrease was 19 %.

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The complement-fixing antibody results were also regrouped to allow comparison with the results of Holzel *et al.* (1953) in England, Yoshino *et al.* (1962) in Tokyo and Halonen (1955) in Helsinki. These results are expressed graphically in Figs. 4–6.

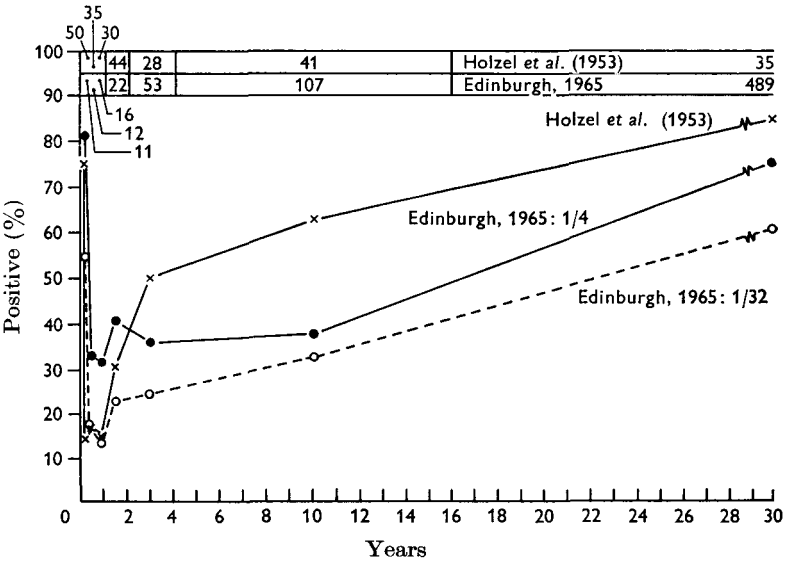

Fig. 4. Comparison of the complement-fixing antibody in Edinburgh and Manchester (Holzel, Feldman, Tobin & Harper, 1955).

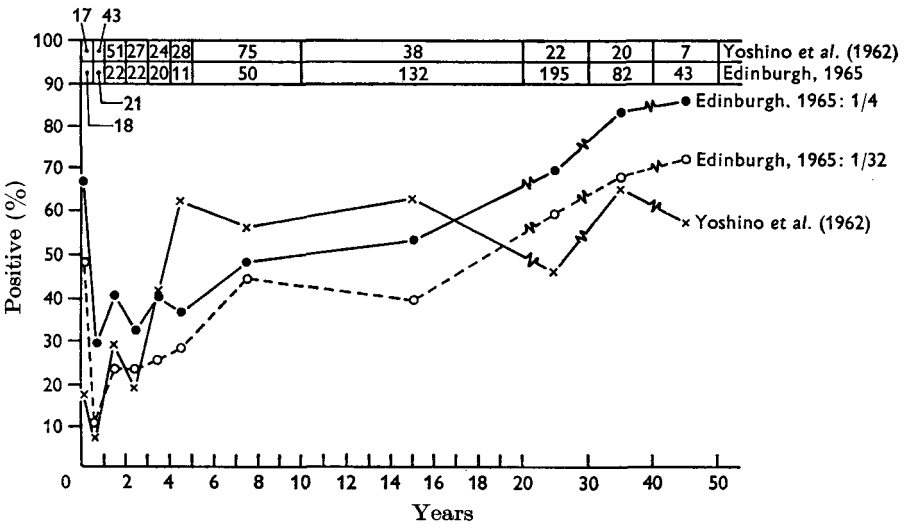

Fig. 5. Comparison of the complement-fixing antibody in Edinburgh and Tokyo (Yoshino *et al.* 1962).

Using the CF (opt.) antigen, the incidence of complement-fixing antibodies in the under-2-year group closely parallels that of Holzel *et al.* (1953) (Fig. 4), but at subsequent age groups there are fewer people with detectable antibody in 1965 (25, 30 and 24% respectively). While the CF (conc.) antigen detects a higher

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proportion of positive sera in the under-2-year-olds, it still shows a lower incidence of antibody in the older age groups (14, 25 and 9% respectively) than that reported by Holzel *et al.* (1953).

Figure 5 shows that the use of the CF (opt.) antigen gives similar results to those of Yoshino *et al.* (1962) over the 4-month to 3-year age groups with an increased incidence when the CF (conc.) antigen is employed. The peak in the 4–5-year group in Japan is not reflected in the Edinburgh results and, in fact, up to the age of 20 years the Edinburgh results are lower than the Japanese ones. After

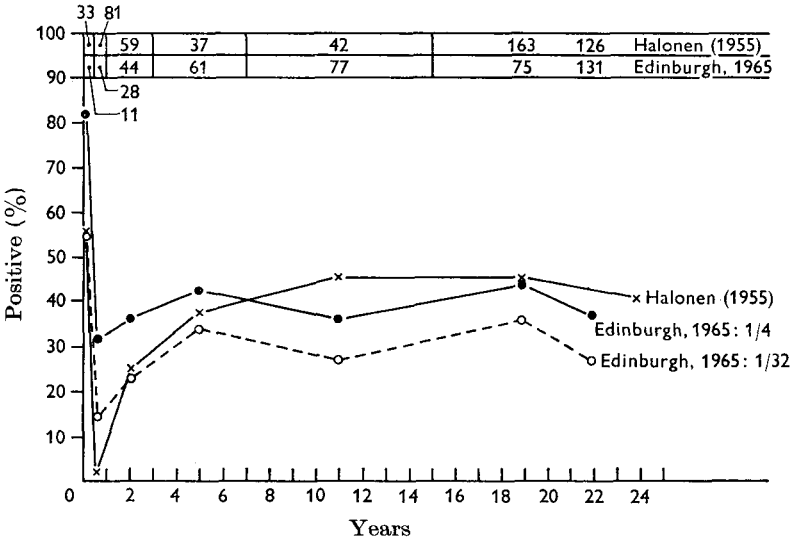

Fig. 6. Comparison of the complement-fixing antibody in Edinburgh and Helsinki (Halonen, 1955).

20 years, however, the incidence of herpes antibody increases in the Edinburgh survey and is in excess of that found in Japan. This may be a reflexion of the numbers in the groups and the techniques employed.

Halonen's (1955) results (Fig. 6) show a very similar trend to those obtained in Edinburgh except in the under-1-year-olds where again, although the Edinburgh CF (opt.) antigen results approach those of Halonen (1955), those of the CF (conc.) antigen give a higher incidence of antibody. In this case it should be noted that over the age of 15, the Edinburgh results for nurses and medical students were used, as they appeared to be more comparable to the conscripts and medical students cited by Halonen (1955).

DISCUSSION

Early workers showed the importance of socio-economic conditions on the incidence of *Herpesvirus hominis* antibodies in sera (Burnet & Williams, 1939). This observation has since been supported by evidence from several other sources. Thus Kibrick & Gooding (1965) in the U.S.A. found that while 84% 'ward service' patients and 62% of private patients had antibody, only 30–37% of college

undergraduates and young doctors possessed any antibody. Becker (1966) compared the incidence of antibody in three populations in Cape Town. He found that in the white population there was a lower incidence of antibody than in the coloured population, but that the Bantus had the highest incidence of antibody.

MacCallum (1959) found that within the 20–50 age group there was a 40–95 % variation in the incidence of antibody found in females in four different communities. These observations are confirmed in Table 3 where it is noted that the Oxford and Edinburgh results for the over-50-year-olds are practically identical (87 and 89 %), whereas those of the younger age groups lie within the 40–95 % limits.

Table 3. *Comparison of the results of antibody studies carried out in Oxford and Edinburgh*

Oxford				Edinburgh			
Year	Age (yr.)	No.	Positive (%)	Year	Age (yr.)	No.	Positive (%)
1962	18–40	18*	66·65	1965	16–43	159*	84
1964	30–49	41†	73·9	1965	30–49	125‡	87
1964	50–69	54†	87·0	1965	50–69	65‡	89

* Antenatal.
† Medical Neurological Service.
‡ Either neutralizing or CF antibody detected.

In this survey, no attempt has been made to classify the people on a socio-economic basis, although it was thought that as the Edinburgh sera and the Oxford adult and antenatal sera were mainly from patients under National Health Service care, there should be no weighting towards the higher socio-economic groups. In addition, the sources of sera employed were very similar to those of previous workers. Acute phase sera submitted for viral diagnostic procedures were used, since such specimens should give a more accurate picture of the incidence of antibody in the general population than convalescent phase sera.

The question of the inclusion of all the nurses and medical students in the survey was considered. As these are both large groups it was felt that they might weight the survey unduly in their approriate sections. Another drawback was that a considerable proportion of the students were from countries other than Scotland, so only the 52 Scottish born and educated medical students were included in the appropriate age groups of the Edinburgh survey.

Apart from the difficulty of background of people included in a survey such as this, there are obvious difficulties in comparing the results of surveys carried out in different parts of the world, at different times, using a variety of antigens and techniques to estimate antibody. Different surveys have employed a number of age groupings, so that subsequent surveys may not always contain an equivalent distribution of people. This defect is obvious in the numbers of Edinburgh sera examined in the under-5-year group, but in this survey emphasis was placed on the 5–25-year group in view of the findings with the medical students and their close similarity to those of Halonen (1955). It will be noted, however, that the

neutralizing and CF (opt.) antigen results in the younger age groups are in agreement with previous findings despite the fewer children in the groups in this survey.

In view of the results of Ashe & Scherp (1963) who found, in neutralization kinetic studies with rabbit antisera, that the laboratory strain HF showed the least cross-reactivity among a number of strains of *Herpesvirus hominis*, a freshly isolated, wild strain of the virus was employed in this survey. In a subsequent publication, Ashe & Scherp (1965) failed to repeat their observation when human serum was used but they did emphasize the difficulty of establishing a base-line in this case. It was, therefore, decided to carry out a small preliminary comparison (unpublished results) using a number of wild strains of the virus isolated in the Edinburgh laboratory. When these were examined by the techniques employed in this survey no distinction could be made between the strain used and the others tested. Similar results were obtained in Oxford.

The close similarity of the incidence of the neutralizing antibodies with that detected by the CF (conc.) antigen suggests that the latter was an efficient antigen for the detection of herpes antibody. When the CF (opt.) antigen was employed, however, the incidence of positive sera was much lower in both the younger and older age groups. This would be expected in the younger age groups as some authors (Murray, Gaon, O'Connor & Mulahasanović, 1965; Grist *et al.* 1966) have reported the necessity of using a concentrated antigen for the detection of primary antibody in sera. The disparity in the older age group may be due in some measure to the quicker decay of the complement-fixing antibody. In this survey 75 % of children under 6 months, in whom the antibody would be largely maternal in origin, had neutralizing antibody, but only 35 % or 17 % had complement-fixing antibodies depending on the antigen employed.

The statement often found in textbooks that 90 % of the population over the age of 15 have neutralizing antibodies was taken from the work of Buddingh *et al.* (1953). This, though an accurate interpretation of the population studied in that paper, does not give a true picture of the situation pertaining today in some other communities in the U.S.A. and in several other countries. Buddingh *et al.*(1953) showed that 85 % of their white children in the 3–14-year age group had neutralizing antibodies, whereas only 41 % of the Edinburgh group had neutralizing antibodies in 1965. In the over-14-year-olds there is a less marked decrease in the incidence of antibody (90–78 %), but as the number of sera examined in 1965 was sufficiently large, it was possible to subdivide this age group. When this was done it was found that the incidence of neutralizing antibody did not exceed 70 % till after the age of 25. Dascomb *et al.* (1955) who, like Buddingh *et al.* (1953) also studied American citizens but from widely scattered areas, found in their series that the incidence did not exceed 70 % till after the age of 30. The sera examined were from air-force personnel and Dascomb *et al.* (1955) suggested that this lower incidence might be due to a high standard of hygiene.

The discrepancy in the incidence of neutralizing antibody in the Edinburgh survey between the 10–14-age group (35 %) and the 15–19-year age group (69 %) seems too great to be due entirely to chance differences in the socio-economic background of the 57 and 75 teenagers respectively on whom these observations

were made. The discrepancy might be correlated with the following clinical observations. First, Jones (1959) has shown that there are two main age groups for the onset of primary ocular herpes, viz. the under 10-year-olds and the 15–25-year age group. Secondly, in Sweden, Eilard & Hellgren (1965) found a steeply rising frequency of herpetic skin infection, in both men and women, after the age of 15 years. This incidence reached a maximum between 20 and 24 years. Lastly, Gold, Stewart & KcKee (1965) recorded a maximum incidence of herpes labialis in the 21–30-year age group. As there does not appear to be any published data concerning the long-term duration of the antibody response in young children following primary herpetic infection it is impossible to determine if this apparent increase in the incidence of herpetic infection in young adults is related to a decrease in the titre of circulating antibody or due to a greater degree of direct oral contact.

Stern *et al.* (1959) recorded the importance of herpetic whitlow as an occupational hazard of nurses, especially in neurosurgical units. These authors reported a 51 % incidence of antibody to herpesvirus in their nursing staff. In this survey, both in Oxford and Edinburgh, there is again a low incidence of antibody in nurses and medical students. A herpetic whitlow has been diagnosed in seven nurses and physiotherapists and one medical student in Oxford in the past 5 years. Thus it would appear that there is still considerable risk of herpetic infection in these young adults in the course of their professional duties.

The CF (opt.) antigen is probably more comparable with the complement-fixing antigens employed by previous workers, but despite using the results from the CF (conc.) antigen, a marked decrease in the incidence was found especially in the comparison with the results of Holzel *et al.* (1953). In the 5–14-year age group there is a decrease of 25 % in the number of people with antibody. Holzel *et al.* (1953) conducted their survey at least 12 years before the Edinburgh one so that children who were 5–14 years old in 1953 would be 17–26 years old in 1965. The incidence of antibody in this group of the 1965 survey was found to be 68 % of the 208 people with the CF (conc.) antigen and 53 % with the CF (opt.) antigen as compared with 63 % in the 1953 survey. Similarly, the 2–4-year age group of the Manchester survey, with a 50 % incidence of antibody, would be equivalent to the 14–16-year age group of the Edinburgh survey which shows an incidence of 53 % positive with the CF (conc.) antigen and 50 % with the CF (opt.) antigen in the 38 people studied.

It therefore appears that there has probably been a decrease in the incidence of herpetic infection in some communities in this country in children compared with 12 years ago, and it would be of interest to determine if this trend has continued in 10 years time. If this is so, there should be a reduction in the number of persons with detectable antibody in the over-30-year age group.

The recent findings of Kapsenberg (1964) and Ross, Subak Sharpe & Ferry (1965) of the antigenic cross-reaction between *Herpesvirus hominis* and varicella-zoster virus do not invalidate the findings of this survey. Acknowledging this cross-reaction, there still appears to be a decrease in the number of people with detectable antibody in certain age groups relative to the number 12 years ago. The

question of whether this antibody was due entirely to infection with *Herpesvirus hominis* or was a result of an anamnestic response to varicella virus applies to all the surveys to date.

This apparent decrease in the incidence of herpetic infection may possibly be explained by improvements in the social environment, especially in improved housing conditions with less overcrowding in homes, coupled with, perhaps, an increased awareness of simple hygienic requirements among greater numbers of the population. Similar factors were suggested by Yoshino *et al.* (1962) to explain the changing incidence of antibody which they found in Japan. Perhaps the similarity of the Edinburgh findings to those of Halonen (1955) also point to an improvement in hygienic conditions in Scotland.

If there is an increase in the proportion of people reaching adolescence with no detectable antibody to *Herpesvirus hominis* it is possible that primary infection of all types (of the skin, mucous membranes and brain) may be seen more often (cf. MacCallum, 1959; Stern *et al.* 1959) in this age group.

SUMMARY

Sera from 1029 individuals, 864 from Edinburgh and 165 from Oxford, have been examined for the presence of antibodies to *Herpesvirus hominis*. The results of the smaller Oxford survey did not reveal a higher incidence of antibody where direct comparison was possible with those from Edinburgh.

The incidence of both complement-fixing and neutralizing antibodies in the sera from 710 people in Edinburgh with ages varying from 1 month to 92 years was compared with the more complete of the earlier surveys, and in particular with that of Holzel *et al.* (1953) in Manchester, England. This comparison revealed a lower incidence of antibody in people under 25 in Edinburgh in 1965.

Results obtained with sera from medical students in Oxford and Edinburgh and from nurses in Edinburgh were in agreement with those of previous surveys. The low incidence of antibody in these young people emphasized the possible occupational risk of infection from patients and that primary herpetic infection might be encountered more frequently than before in teenagers and young adults.

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