Serological studies in a student population prone to infection with human papilloma virus

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SUMMARY

Four hundred and sixty-seven serum specimens from the female students at a residential college were examined for the presence of circulating antibody to human wart virus using the technique of counter-current immunoelectroosmophoresis. A significantly higher incidence of antibodies was found in students with a past history of plantar warts than in any other group. Antibody took several months to develop and was detectable in 20–30 % of the students up to 9 years after infection. From a few cases of multiple infection, it was shown that reinfection could occur in spite of the presence of circulating antibodies probably of the IgG class. The sensitivity of the test was compared with two recognized techniques for detection of wart virus antibodies, namely gel diffusion and passive haemagglutination.

INTRODUCTION

The high incidence of warts and especially of plantar warts in school children and young adults is well recognized to be associated with the increasing availability of swimming baths (Hall & Burrows, 1968; Allen & Dickenson, 1968; Tranter, 1969). Plantar warts may pass undiagnosed for several months, and they are more often painful and disabling than other types of warts, but it has been considered that one attack may provide protection from subsequent infection. A study into the duration and immunizing properties of the antibody produced after infection has been carried out over a period of 4 years on the students at a girls' residential College of Physical Education, with its own swimming pool and, until recently, a high incidence of plantar warts (Bunney, 1971, 1972).

Several tests for the detection of circulating antibody to human papilloma virus have already been described (Almeida & Goffe, 1965; Ogilvie, 1970*a*). The passive haemagglutination test of Ogilvie is a sensitive method, particularly for the detection of antibodies of the IgM class. It is, however, fairly laborious, technical details are important and there is as yet the difficulty of establishing a base-line for significant titres. This is demonstrated by the fact that 21 out of 34 (61·8 %) of girls professing never to have had warts showed titres ranging from 20 to 320; nine of these girls with titres of 80 or above were considered to have significant antibody titres (Ogilvie, 1970*a*). The direct agglutination test described by Almeida and Goffe is relatively extravagant in materials, and the formation of precipitin

lines in agar gel diffusion is a rather insensitive method. It was, however, felt that a method which could increase the sensitivity of the precipitin reaction would be quick and useful for screening fairly large numbers of sera. The technique of counter current immunoelectroosmophoresis has been utilized to detect a variety of antigen-antibody interactions. In 1959, Bussard reported its use with snail haemocyanin and *E. coli* as antigens and mentioned, without giving details, that the technique had been tried successfully elsewhere with virus and homologous antibody. More recently, it has been shown to be an effective method in the detection of serum hepatitis antigen (Pesendorfer, Krasnitsky & Wewalka, 1970) and its increased sensitivity over gel diffusion and practicability of use when samples are small or numbers of specimens large has been demonstrated (Prince & Burke, 1970).

MATERIALS AND METHODS

Population under study

At the beginning of the 1970 and 1971 academic sessions, small samples of blood were obtained from the majority of first-year students. Earlier specimens were collected with the aid of the Blood Transfusion Service which visits the College of Physical Education at 6-monthly intervals. The students donating blood were asked to give a small extra sample at the same time. This meant that of the 1968 and 1969 intakes at the College a smaller number of students were tested, but from many of them two or three samples were obtained at different times. Sera were separated from the clotted bloods and stored at -20°C . Each girl in each year completed a questionnaire requesting details of wart history. The same histories were used by Bunney (1971, 1972) in a trial of protective footwear. In 1971 a more comprehensive history giving more details of each attack and any treatment received was obtained.

Technical methods

Preparation of antigen

Parings from the surface of simple plantar warts were obtained from patients attending the wart clinics at the Skin Department of the Royal Infirmary, Edinburgh, collected in saline containing 200 units penicillin and 200 μg . streptomycin per ml. and stored at -70°C . When a large amount of material had been obtained it was pooled, chopped with scissors and finely ground in a mortar. The extract was clarified by slow centrifugation and then semi-purified by two cycles of ultracentrifugation, the first at 9200 g for 15 min. and the second at 58,000 g for 90 min., in a Spinco Model L ultracentrifuge. The pellet from the second cycle was resuspended in a small volume of saline. The number of particles in the extract was obtained by counting a negatively stained preparation in the electron microscope, with reference to a known latex suspension, using the method of Watson, Russell & Wildy (1963). The antigen preparation was then diluted in saline for use in the electrophoresis tests. Control antigens included an extract of plantar callouses and an extract of a contaminating yeast isolated from one of the wart virus preparations.

Fig. 1. Templates used in IEOP Tests: (a) To test forty serum samples on $3\frac{1}{4} \times 3\frac{1}{4}$ in. slides, (b) and (c) to enable reactions of identity to be detected.

Antisera from rabbits immunized with extracts of human papilloma virus were kindly provided by Dr M. M. Ogilvie.

Immunoelectroosmophoresis (IEOP)

Cleaned glass plates ($3\frac{1}{4} \times 3\frac{1}{4}$ in.) were coated with 5 ml. of 0.9% agarose in distilled water. This was allowed to dry down to a thin film, after which 30 ml. of 0.9% agarose in 0.025 M barbitone buffer, pH 8.6, were applied to the slide held on a levelling tray. A template was used to cut pairs of holes, 2 mm in diameter and with a centre to centre distance of 7 mm. (Fig. 1 (a)), thus enabling forty samples to be tested simultaneously, usually as twenty serum specimens with two concentrations of antigen or two different antigenic extracts. Occasionally, microscope slides (3×1 in.) were used, as for example in identity reactions. These were prepared in the same way, using 16 ml. of coating agar and 50 ml. of top agar for batches of eight slides held in a Shandon slide tray.

One of the initial disadvantages of the IEOP test was the inability to produce reactions of identity. Das, Hopkins, Cash & Cumming (1971), however, described a modification of the technique, using a different template consisting of two antigen wells equidistant from a single serum well, which was capable of detecting reactions of identity, partial and non-identity. The template found most satisfactory for identity reactions in this study consisted of a triangle of wells with centre to centre distance of 7 mm, with the serum wells 2 mm in diameter and with the antigen well 5 mm in diameter (Figure 1 (b)). A system of wells and trough (Fig. 1 (c)) was also used but much greater difficulty was experienced in obtaining complete continuity of the precipitin lines.

The serum wells were filled and the plate placed in a Shandon electrophoresis tank fitted with a cooling platen and containing 800 ml. 0.05 M barbitone buffer, pH 8.6. Wicks of glass fibre paper were used to complete the circuit between plate and buffer.

A constant current of 25 mA. was applied with the polarity such that the

serum was always on the positive side, and the current was run for 10 min. The plate was removed, the serum wells topped up and antigen added to the appropriate well. The plates were re-exposed to a current of 25 mA for 1 hr. after which precipitin lines could be examined by viewing with a bright light against a dark background. The plates were always incubated overnight at 37° C. in case further lines developed.

The plates were normally preserved thereafter by staining as follows: they were washed overnight in several changes of isotonic saline to remove unprecipitated protein. They were then allowed to dry at 37° C, covered with a sheet of filter paper for 4–5 hr., before immersing in 0.2 % Ponceau S in 5 % trichloroacetic acid (TCA) for 3 hr. Excess stain was removed by three washes in 5 % TCA followed by a quick rinse in distilled water. The plates were allowed to dry in air. Occasionally, precipitin lines were highlighted by the technique of Alpert, Monroe & Schur (1970) by immersing the plate in 1 % tannic acid after the saline washes, leaving for 15 min. and examining directly. Precipitin lines were much more easily observed by this method, but it was only suitable when permanent records were being made by photographing the plates, since staining after tannic acid treatment was not wholly successful.

Determination of immunoglobulin class of antibody

Heavy molecular weight immunoglobulin (IgM antibody) was destroyed using 0.1 M 2-mercaptoethanol (2-ME) in saline with pH adjusted to 7.4. Equal volumes of 2-ME and serum were mixed and held for 24 hr. at 4° C. Before running in the IEOP test, whole sera and 2-ME treated sera were diluted 1/2 and the test carried out as usual, using the mixture and its dilution. The presence of 2-ME did not interfere with the test.

Agar gel diffusion and passive haemagglutination tests

The technique used for both these methods were essentially those described by Ogilvie (1970*a, b*). In the gel diffusion test, 0.8 % agarose was sometimes used and this was diluted in PBS pH 7.2 or in 0.025 M barbitone buffer, pH 8.6, the latter giving a more direct comparison with the IEOP result.

RESULTS

Antigen concentration

The concentration of antigen in an IEOP test is important. The extract must contain a very large number of particles, but antigen excess can prevent the development of precipitin lines in the area between the wells when tested against a weakly positive serum. On the other hand, a concentration which can detect such sera does not seem to obscure more highly positive sera. Furthermore, antigen excess can cause the production of two precipitin bands or a diffuse drawn-out band. For each antigen extract a small titration was carried out, using a range of sera shown to be positive by precipitation in gel diffusion, and the optimum concentration calculated. Although the dilution factor required to reach this concentration varied from preparation to preparation, the number of particles

Table 1. *Comparison of the techniques of simple gel diffusion and IEOP for the detection of antibody to human wart virus*

Year of collection	No. of specimens tested	IEOP results	Gel diffusion results	
			No. positive	No. negative
1968	55	18 positive	6	12
		37 negative	1	36
1969	40	12 positive	7	5
		28 negative	0	28
1971	36	31 positive	25	6
		5 negative	0	5

Table 2. *Comparison of PHA titres with the results obtained by gel diffusion and IEOP in the detection of antibody to human wart virus*

PHA titre	Gel diffusion, % + ve results	IEOP, % + ve results	No. of specimens tested
10 or less	7.7	23.1	13
20	0.0	16.6	12
40	0.0	46.7	15
80	16.6	50.0	18
160	16.6	100.0	6
320 or greater	100.0	100.0	6
Totals	15.7	47.1	70

present was remarkably consistent, e.g. 1.8×10^{11} and 1.4×10^{11} particles/ml in two different preparations. It was found necessary to dilute the antigen in saline rather than in the barbitone buffer since the latter caused the potency of the extract to fall after several freezing and thawing cycles and the particles of papilloma virus to be broken down (Plate 1).

Comparison of IEOP and other serological methods

The increased sensitivity of the IEOP test over simple gel diffusion in agar was shown by direct comparison of 131 samples. Considerably more positive results were obtained by IEOP (61 to 39, see Table 1) and these figures are significant at the 1.0% level. The 1971 sera which were positive by IEOP were then compared with gel diffusion tests using agarose instead of agar, dissolved in both PBS and barbitone buffer. The results were consistent (25/31 positive in gel diffusion) with one exception – a serum weakly positive by IEOP gave a precipitin line with barbitone-buffered agarose but not with PBS-buffered agarose. No precipitin lines were obtained in IEOP with the antigen from plantar callouses and only one serum gave a weak precipitin line with the yeast antigen.

The IEOP and gel diffusion tests were also compared with the passive haemagglutination (PHA) test. Of the sera used in the present survey, 55 specimens collected in 1968 had previously been tested by Dr M. M. Ogilvie and her PHA and gel diffusion results were made available. A few further PHA and gel diffusion

Table 3. *No. of specimens received from each year*

Year	1st specimens	Repeat specimens	Total
1968	82	49	131
1969	50	21	71
1970	107	49	156
1971	109	—	109
Total	348	119	467

results were obtained from sera collected in 1969. The results of these comparisons are shown in Table 2, where it can be seen that almost 50 % of sera with PHA titres of both 40 and 80 were detected by IEOP, but a titre of greater than 160 was required to ensure detection by gel diffusion.

Specificity of IEOP

It has been possible by means of the IEOP system to obtain reactions of identity between human and human serum, rabbit and rabbit serum and human and rabbit serum against the same antigen using the template shown in Fig. 1(a). It was found that a lower concentration of antigen was required to obtain reactions of identity than in straightforward IEOP, presumably because the volume of antigen applied to the large well was greater than normal. Furthermore, reactions of identity with asymmetric arcs (Ouchterlony, 1964) were often obtained when the difference in amount of antibody led to a slight imbalance in the system.

IEOP survey of antibody to human papilloma virus

Specimens of sera were obtained from the girls of the college in the numbers shown in Table 3. No follow-up of the 1971 students has as yet been possible.

The students had been placed in seven groups according to their wart histories for the investigation of the value of protective footwear in preventing the spread of wart infection (Bunney, 1971, 1972). These are shown in Table 4, together with the percentage of students showing antibody in each group, as determined from the first serum samples obtained in each case. Those showing a past history of plantar warts were found to have a significantly higher ($P < 0.0005$) level of antibody than those in other groups. The presence of antibodies in some of the girls who said they had never had warts will be discussed.

Development of antibody

As foot inspections were carried out at about 6-monthly intervals at the college and as repeat histories were obtained with second and third samples of serum, it was possible to study the development of precipitating antibody in students with warts present at the time of sampling. The results are shown in Table 5. Although eighty-one specimens were available with all the relevant information, the number in each group was rather small. However, it would appear that most people did not develop antibody to wart virus until the infection had been present for a few months. The overall results for the two sections, namely, plantar warts and other

Table 4. *Group analysis of sera tested by immunoelectroosmophoresis for the presence of circulating antibody to human wart virus*

(All first serum samples from 1968-71 student intakes.)

Group	History	Year	No. of specimens		% positive	Proportion positive	%
			Tested	Positive			
I	Past history, plantar warts	1968	29	12	44.3	51/112	45.5
		1969	11	3			
		1970	20	7			
		1971	28	17			
II	Past history, plantar and other warts	1968	9	4	50.0	19/75	25.3
		1969	1	0			
		1970	12	6			
		1971	2	2			
III	Past history, hand warts	1968	11	3	25.8	17/94	18.1
		1969	14	5			
		1970	15	2			
		1971	22	6			
IV	Past history, other warts (legs, face etc.)	1968	4	2	23.0	19/67	28.4
		1969	1	0			
		1970	4	1			
		1971	4	0			
V	No past history of warts	1968	15	2	18.1	19/67	28.4
		1969	17	5			
		1970	35	4			
		1971	27	6			
VI	Present history of warts other than plantar	1968	10	2	31.8	19/67	28.4
		1969	5	2			
		1970	8	1			
		1971	21	9			
VII	Present history of plantar warts	1968	4	1	21.7	19/67	28.4
		1969	1	0			
		1970	13	3			
		1971	5	1			

types of wart, were remarkably similar, and it would seem that a second exposure to virus, whatever the site of earlier exposure, was more likely to result in antibody formation.

Duration of antibody

Figure 2 represents in histogram form the duration of antibody as determined from 156 samples from students showing a past history of plantar warts and from 99 samples from those who had had other types, mainly hand warts. No samples were obtained from anyone who had had plantar warts more than 9 years before and only two girls had a history of hand warts less than a year ago. It can be seen from the histograms that the likelihood of developing circulating antibody was slightly greater following plantar wart infection, and once formed, antibody persisted in about 35 % of plantar wart patients for several years, but in less than 20 % of hand wart cases after five years. Antibody was, however, detected in one

Table 5. *Development of antibody during wart virus infection*

Type of wart present	Previous exposures to virus	Duration of lesion in months												Total specimens examined	
		< 2		2-6		6-12		12-24		> 24					
		No. tested	No. +ve	No. tested	No. +ve	No. tested	No. +ve	No. tested	No. +ve	No. tested	No. +ve	No. tested	No. +ve	No. tested	No. +ve
Plantar	None	—	—	3	3	3	1	—	—	—	—	6	4		
	One or more	9	1	7	2	5	2	2	1	—	—	23	6		
	Total	9	1	10	5	8	3	2	1	—	—	29	10		
Others mainly hand	None	1	0	3	2	4	1	3	1	4	2	15	6		
	One or more	5	2	10	5	6	4	5	3	11	0	37	14		
	Total	6	2	13	7	10	5	8	4	15	2	52	20

Fig. 2

Table 6. *Change in antibody levels without change in wart history in individual students*

Serum	Group	Detailed history at time of 1st sample	Ab results	Ab results × months later			Outcome
			1st sample	6	12	18	
E33	I	P 4	+	.	.	-	Ab lost in 4-5 years
D210	I	P 12, 4, and > 1	++	+	.	.	Ab falling in < 1 year
D205	II	P < 1, H 10	++	+	.	.	Ab falling in < 1 year
D67	II	P 2, E 10,	+	.	-	.	Ab lost in 2-3 years
D84	IV	E 9	+	+	.	-	Ab lost in 10-11 years
F110	VI	P 3, H present 3/12	-	+	.	.	Ab gained in 3-9 months
F43	VII	P 5, H present 3/52	-	.	+	.	Ab gained in < 12 months

Abbreviations: groups as in Table 2; P = plantar wart, H = hand wart, E = wart elsewhere; number following = no. of years since wart, e.g. P 2 = 2 years since plantar wart, Ab = antibody; . = not tested.

case as long as 13 years after a history of hand warts (the warts had apparently lasted only about two months and had received no treatment).

It was possible to watch the change in immunological state of a few of the students from whom two or more serum samples were obtained while the wart history remained unchanged. These are detailed in Table 6 and fit in with the general picture shown in Table 5 and Fig. 2. Sera D210 and D205 were tested for the class of immunoglobulin present. Both samples of D210 were shown to contain mercaptoethanol-resistant antibody following treatment with 2-ME; neither sample of D205 showed any precipitin lines after 2-ME treatment. Immunoglobulin class was not tested in the remaining sera shown in Table 6.

Table 7. *Antibody levels following repeated plantar wart infections*
(Abbreviations as in Table 6.)

Serum no.	Group	1st serum specimen			2nd serum specimen			3rd serum specimen		
		Detailed history	Ab result	2ME resistant Ab	Detailed history	Ab result	IgG	Detailed history	Ab result	2ME resistant Ab
D 17	I	P 6 and 1	+	+	P 6½ and 1½	+	+	P 8, and < 1	+	+
D 47	I	P 7 and 3	+	+	P 9, 5 and 1	-	.	.	.	.
D 53	I	P < 1	+	-	P 3 and < 1	-	.	.	.	.
D 74	I	P 3½	-	.	P 5 and present 1/12	-	.	P 5 and < 1	-	.
D 113	I	P 6½	+	+	P 8 and present 6/12	+	+	.	.	.
D 121	II	P and E 1, H 8	-	.	P and E 3, H 10, P present 3/12	-	.	P and E 3½ H 10½ P < 1	-	.
E 28	II	P and H 7	-	.	P and H 9, H present 12/12	-	.	.	.	.
F 93	II	P 3 and H 7	+	-	P 3½ and H 7½	+	-	P 4, H 8, H present 3/12	+	+

Table 8. *Antibody response in treated and untreated warts*

Group		Plantar warts				Hand and other warts					
		Groups I and II		Group VII	Groups III and VI		Group VI		Totals		
		Ab + ve	Ab - ve		Ab + ve	Ab - ve	Ab + ve	Ab - ve			
		Ab + ve	Ab - ve	Ab + ve	Ab - ve	Ab + ve	Ab - ve	Ab + ve	Ab - ve		
Treated	No. specimens tested	14	11	1	2	4	7	3	2	22	22
	Average duration in months	4.1	4.4	—	—	16.2	8.4	—	—	—	—
Untreated	No. specimens tested	4	0	0	0	2	13	6	10	12	23
	Average duration in months	6.0	—	—	—	4.0	6.0	—	—	—	—

Antibody titres after repeated attacks of warts

The variation in the response of girls with repeated histories of plantar warts is shown in Table 7. As might have been expected, the girls subject to repeated attacks would appear to have developed antibody and lost it quickly or not to have developed it at all. The probable presence or absence of IgG in the sera was therefore determined by mercaptoethanol treatment. It can be seen that reinfection could occur in spite of the probable presence of IgG antibodies (girls with serum specimen numbers D 17, D 47 and D 113). With serum D 53, and the first two samples of F 93, the mercaptoethanol treatment destroyed the precipitin-producing antibody, suggesting that it belonged to the IgM class, although in the latter case, this would be a very long-surviving IgM. In the remaining three cases, no circulating antibodies were detectable at 1, 3½ and 7 years after primary infection and no antibodies were present in any of these students within one year of the most recent lesions.

The effect of treatment

In the 1971 questionnaire, students were asked to give details of the duration of their warts, past as well as present and of any treatment given. None had had their warts removed by curettage, but various chemicals ranging from the commercially available 'Compound W' and formalin to more orthodox treatments such as podophyllin, wart paint and liquid nitrogen had been used. Table 8 shows that more people with plantar warts received treatment than did those with hand and other warts, and that the application of a chemical remedy did not prevent the development of antibody, a finding noted by Bunney, Hunter, Ogilvie and Williams (1971). The presence of antibody in some members of the treated group did not appear to influence the duration of the wart, although the groups tested here were too small for critical analysis.

DISCUSSION

It has been shown by Ogilvie (1970*b*) that plantar warts more often contain sufficient virus to be detected in the electron microscope than other types of wart. This fact, together with the pressure of the body weight on a plantar wart, suggests that the antigenic stimulus would be greater in such warts. It might therefore be expected that more people with plantar warts than with hand warts would develop antibody to the virus. A figure of 18.5% positive in a group of girls professing never to have had warts (Table 4) is disappointing, but plantar warts can occur and resolve spontaneously without recognition. Furthermore, it is surprising how often warts of many years ago are forgotten. Each intake of students was given a lecture about wart virus infections before filling in the form to minimize such errors but, with the 1969 intake, the lecture was given sometime after the first serum samples were collected. About forty girls therefore filled in two forms and 25% of them gave different histories on the two occasions! While the lack of complete consistency with histories obtained directly from the girls may need further clarification experimentally, it seems more likely that questioning the girls

individually would have revealed many forgotten instances of infection (Rasmussen, 1958). Examination of sera taken at intervals following clinically confirmed infections would give better correlation, and this is at present being pursued.

The results showing the development of antibody during wart virus infection (Table 5) suggest that antibody developed to an almost equal extent in plantar and other wart infections, provided there had been an earlier antigenic stimulus. On the other hand, with the first exposure to virus, antibody appeared to develop more readily if the infection occurred on the feet. Although the time taken for antibody to develop appeared to be similar for plantar and non-plantar warts, the former tend to be recognized more quickly because of the pain which often accompanies them and because of the regular foot inspections held in school and college swimming baths. This may mean that the duration of the hand warts reported here has been underestimated.

Eight out of fourteen students continued to show antibody up to a year after plantar warts had disappeared (Fig. 2). A further nine out of ten showed antibody during the period 1–2 years after infection, giving a total figure of 70·8% in the first two years. Although Ogilvie (1970*a*) showed precipitating antibody to be present in all of seven patients tested 3 months to 2 years after cure, Bunney *et al.* (1971), in a controlled treatment trial, found that only 70·5% (24 out of 34) patients whose warts were cured within a 12-week period showed an antibody response at the time of cure. The present survey has shown in addition that the percentage of people continuing to display antibody remained fairly high for 8–9 years, particularly following plantar wart infection.

In both the rabbit papilloma (Kidd, Beard & Rous, 1936) and bovine papilloma (Lee & Olson, 1968) systems, the sera from tumour-bearing animals has been shown to be capable of neutralizing the virus *in vitro* and preventing successful reinoculation. In a series of calves inoculated with bovine papilloma virus, Lee & Olson (1969) found that a precipitating antibody, IgG in nature, was consistently produced. Although IgG antibodies are normally considered to be protective, these workers did not always observe resistance to reinfection. It would appear from the results in Table 7 that a similar situation might exist with human papillomas – new warts developed in three cases in spite of the presence of mercapto-ethanol-resistant antibodies.

The nature of the antibody response to human wart virus has been previously examined (Goffe, Almeida & Brown, 1966; Ogilvie, 1970*a, b*). Goffe *et al.* showed 72·2% (13 out of 18) patients to have IgM antibodies, two to have IgG only and three to have both types of antibody, thus suggesting that not many of this group were protected. On the other hand, Ogilvie (1970*b*) showed 75% (21 out of 28 patients) with simple plantar warts to have IgG at the time of cure as opposed to 60% (29 out of 49) for all types of warts. In addition, only four out of ten of a group of mosaic wart patients had IgG, again supporting the idea that simple plantar warts offer a greater antigenic stimulus. Possibly if the amount of virus was further increased, as in the above-mentioned results of Lee and Olson with experimentally inoculated calves, IgG would always be produced.

While it is unlikely that antiviral antibody plays any part in the cure of a wart,

it is apparent from the results reported here that some cases of resolution, both spontaneous and following treatment, do develop antibody. The regression of a virus-induced tumour has been shown in many cases to be due to a cell-mediated immune response (Shope papilloma – see Kidd *et al.* 1936; Evans, Weiser & Ito, 1962; Kreider, 1963; bovine papilloma – see Lee & Olson, 1968; rabbit fibroma and other viruses – see Allison, 1967). In particular, Lee & Olson (1968) have shown that existing bovine papillomata continue to grow after humoral antibody has developed and then regress simultaneously with lymphocyte infiltration being a characteristic histological finding. In humans, simultaneous regression of large crops of warts has frequently been observed (Rowson & Mahy, 1967) and a similar method of destruction seems likely.

On a quantitative basis, IEOP was shown by Prince & Burke (1970) to give titres ten times higher than standard Ouchterlony gel diffusion. They also showed that all sera positive by gel diffusion were positive by IEOP, but the latter method detected a significantly higher proportion of positives. While no comparative titrations were done with sera in the present survey, the results were similar – a single specimen was detected by gel diffusion and not by IEOP, while the overall difference in number of positives detected by IEOP was significant at the 1.0 % level (Table 1).

The present results confirm the usefulness of the technique of immuno-electrophoresis, particularly in studies involving large surveys, where speed, ease and sensitivity are important considerations. Much remains to be learnt about wart virus serology, but the techniques described above should make prospective, comprehensive studies feasible.

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EXPLANATION OF PLATE

Human papilloma virus particles: (A) in saline, (B) partly degraded by several cycles of freezing and thawing in a 0.05 M barbitone buffer. $\times 80,000$.