J. Hyg. Camb. (1970), **68**, 479
With 2 plates
Printed in Great Britain

Serological studies with human papova (wart) virus

By MARIE M. OGILVIE
Department of Bacteriology, University of Edinburgh

(*Received* 6 *April* 1970)

SUMMARY

Methods for demonstrating antibody to wart virus by complement fixation and passive haemagglutination tests are described and compared with the precipitin test of Almeida & Goffe (1965). The results reveal the much greater sensitivity of the passive haemagglutination method, particularly in the detection of the immunoglobulin M class of antibody. Both complement-fixing and precipitating antibody were detected in sera from patients whose warts had undergone a spontaneous resolution.

The presence of antibody to wart virus was demonstrated in sera from persons who had had warts up to 10 years previously, and in a few cases from those who thought they had never had warts.

The antigenic identity of virus from hand warts and plantar warts of the simple and mosaic types was revealed, and some evidence was obtained for similar identity of the virus from genital warts.

INTRODUCTION

One human tumour that is without doubt due to a virus is the simple wart (*verruca*) or papilloma. This human wart virus is classified in the PAPOVA group (Melnick, 1962) along with other small DNA-containing oncogenic viruses. Much useful knowledge of the role of viruses in neoplastic processes has come from study of two animal viruses, SV 40 and polyoma, also members of the papova group. Research concerned with the activity of the human papova (wart) virus has, however, been severely limited because of the continuing lack of any system for propagating the virus; yet it has much potential value as a model for viral oncogenicity in man.

Electron microscopic studies have revealed the presence of typical papova viruses in various forms of wart—the types commonly seen on the hands and feet (Almeida, Howatson & Williams, 1962), papillomata of the oral mucosa (Frithiof & Wersall, 1967), and condylomata acuminata or genital warts (Dunn & Ogilvie, 1968). Other reports of similar particles, found in human tissues significantly associated with malignant changes, include that of Ruiter & van Mullem, (1966) regarding the lesions of epidermodysplasia verruciformis, and a recent description of the particles in certain cells cultured from a nephroblastoma (Smith, Pinkel & Dabrowski, 1969). There is clearly a need for methods of identifying these viruses more accurately and for studying the host response to them.

Almeida & Goffe (1965) described techniques whereby antibody to wart virus

could be detected in sera, using either (i) direct agglutination, observed in the electron microscope, or (ii) the formation of precipitin lines in agar gel diffusion tests. Sera from 45 % of their 42 patients with warts were found to contain antibody detectable by these methods. In addition, in 13 out of 18 the antibody was entirely of the immunoglobulin M class that does not confer immunity (Goffe, Almeida & Brown, 1966).

The aim of this project was to examine the nature and development of the antibody response to wart virus more fully, and to study its relationship to regression of warts. It was hoped that the antigenic properties of virus from the different types of wart could be examined, and more sensitive methods for demonstrating antibody to the virus found.

MATERIALS AND METHODS

Virus antigens

Wart tissues were collected in saline containing antibiotics (penicillin 200 units/ml. and streptomycin 200 μg./ml.). Material from warts of any one type was pooled, minced finely with scissors and ground in a mortar. The extract was clarified by differential centrifugation, the pellet obtained after ultracentrifugation, (at 100,000g for 90 min.) being resuspended in a small volume of distilled water and used as the antigen.

Electron microscopy

Negative staining was done with 2 % sodium phosphotungstate, pH 7·2. Some antigen preparations were seen to contain large numbers of wart virus particles, and these were counted with reference to a latex suspension of known concentration, using the loop drop method of Watson, Russell & Wildy (1963). Agar blocks cut out from gel diffusion tests were dissected out in a drop of distilled water to which a coated specimen grid was applied and the adherent drop then negatively stained.

Rabbit sera

Two rabbits (Nos. 1, 2) were inoculated with the antigen from simple plantar warts. Rabbit 1 received one subcutaneous dose of antigen emulsified in an equal volume of oil–Arlacel adjuvant, according to the method of Herbert (1967*b*), followed by one intravenous dose, without adjuvant, 10 weeks later: rabbit 2 had three intravenous doses at weekly intervals and a fourth 8 weeks after the third.

A third rabbit was given a cellular antigen prepared as an approximately 10 % suspension from genital wart tissue by passing it through a fine-mesh sieve (Evans, Gorman, Ito & Weiser, 1962). The first inoculation was of 2 ml. intradermally, the second 0·7 ml. subcutaneously, and a final 1 ml. intravenously, with some months between each. Sera were obtained from all the rabbits before and after the inoculations.

Human sera

Sera were obtained from adult patients attending Wart Clinics at the Royal Infirmary, Edinburgh. When possible serial samples were taken. Some sera were also obtained from girls at a local residential college where a survey into wart infection was carried out. The girls were aged 17–23.

All sera were stored at −30° C. and inactivated by heat at 56° C. for 30 min. before use in tests except for the precipitin test, in which they were examined without inactivation.

Precipitin tests

Microscope slides (3 × 1 in.) were coated with 2 ml. of 1 % Ionagar No. 2 (Oxoid) in distilled water and dried. A top layer of 2 ml. 0·7 % agar in phosphate-buffered saline pH 7·2 was set on the precoated slides and wells cut with a diameter 2 mm. and separated from a central well by distance of 3 mm. Tests were incubated in a humidified atmosphere at 37° C. and read at 24 and 48 hr. for the formation of precipitin lines. When required, agar was poured in Petri dishes and larger wells cut, (diam. 5 mm. set 7 mm. apart). These tests were read after 3 days at 37° C. and the precipitin lines recorded photographically using dark ground illumination.

Complement fixation tests

The standard method of Bradstreet & Taylor (1962) was used, with overnight fixation at 4° C. and 3 HD50 of complement. Optimal concentration of antigen was determined by 'chessboard' titration against antiserum to wart virus, and the complement was titrated in the presence of antigen at this concentration. Perspex plates were used, and 0·1 ml. unit volumes. An uninfected control antigen preparation was made from parings of simple callouses in the same way as wart antigen, but the clarified extract was used without ultracentrifugation as no pellet was obtained.

Passive haemagglutination tests

The method used was essentially that of Herbert (1967*a*). Fresh sheep red cells were tanned by incubating a 5 % suspension of cells with an equal volume of tannic acid (BDH) solution, containing 1 mg. in 10 ml. phosphate-buffered saline pH 7·2, at 37° C. for 15 min. The tanned cells were coated with virus antigen by incubating 3 vols. of the 5 % tanned cells in PBS pH 6·4 with one volume of virus antigen and a further 2 vols. of PBS pH 6·4. The optimal amount of antigen for sensitization was previously determined from observation of the concentration that gave the highest titres in sera when several different concentrations were used to coat separate samples of cells. The sensitization had to be carried out at pH 6·4 and not the 7·2 used for other steps in the procedure, and an incubation time of 30 min. at 37° C. was satisfactory. Once sensitized, the cells were washed three times in PBS containing 1 % normal rabbit serum as stabilizer (NRS diluent) and were resuspended in it at a final cell concentration of 1 %.

Sera to be tested were first adsorbed with sheep red cells from the same batch

in order to remove any heterophile agglutinins. A 1/10 dilution of serum was made in a 1 % suspension of the cells and left at room temperature for 30 min. The cells were then removed by centrifugation and the serum inactivated at 56° C. for 30 min. Tests were carried out in disposable plastic plates with conical-bottomed wells (Linbro Chemical Co.), using 0·025 ml. unit volumes. Doubling dilutions of sera were made in the NRS diluent with micro-diluters (Cooke Engineering Co.) and the sensitized tanned cells added to these wells. Controls consisted of the lowest serum dilution plus unsensitized tanned cells, and both sensitized and unsensitized tanned cells alone in the diluent. Tests were left at 4° C. overnight before the pattern of the sedimented cells was read. Serum titre was taken as the highest dilution showing any haemagglutination.

Immunoglobulin determinations

(i) Mercaptoethanol treatment was used to destroy the immunoglobulin M. Serum was incubated with a $\frac{1}{10}$ volume of 0·2 M 2-mercaptoethanol (2-ME) at 37° C. for 1 hr. An equal volume of saline was added to an untreated sample of the serum. Such sera were examined directly in the precipitin and passive haemagglutination tests as the presence of the 2-ME did not appear to interfere with either test.

(ii) Some sera were fractionated on sucrose density gradients to allow the distribution of antibody in the fractions to be determined. Gradients prepared from 40 to 10 % sucrose were left to equilibrate overnight. Using a 1/2 dilution, 0·5 ml. of serum was layered on top and centrifuged at 35,000 rev./min. for 16 hr. in the SW 39 rotor of the Spinco model L. The fractions of 0·48 ml. were collected from the top of the tubes. With each run, a serum containing Paul Bunnell heterophile antibody was included, and a check on its distribution made by doing a Paul Bunnell test on the fractions (Cruickshank, 1965). This precaution, introduced at the recommendation of Pinckard (personal communication), gave a standard for the level where immunoglobulin M should be found in the fractions, as the heterophile antibody belongs to this heavy class (Carter, 1966). The wart sera fractions were examined directly in complement fixation and passive haemagglutination tests as sucrose in the amounts present did not interfere apparently with either test.

RESULTS

Precipitin tests

As had been found by Almedia & Goffe (1965), no antibody was detected by precipitin test in sera from many patients with warts. This is a relatively insensitive test, sera have to be examined undiluted and minimum antigen concentrations of the order of 10^{11} particles/ml. are required for the formation of readily visible precipitin lines. In spite of this, it was possible to demonstrate the appearance of antibody during the course of a wart infection in 78 out of 116, i.e. 68 %, patients whose sera were examined around the time of cure of their warts (Table 1). The 14 who had antibody in early sera are included in this number. In addition it can be seen that in the 147 cases whose sera were examined only early, before there

was any evidence of regression, precipitating antibody was found in only 14, i.e. 10 %. Twenty-seven people were shown to have antibody in a second serum when there had been none in their first. In eight others, increased amounts of precipitin were noted in the second serum, and the replacement of the 2-mercaptoethanol sensitive immunoglobulin M by resistant immunoglobulin G could be seen. The longest period over which any one patient was found to have persisting IgM was 18 months, and it was found on several occasions during that time that there were virus particles to be seen in parings from her mosaic plantar wart.

Table 1. *Incidence of precipitating antibody to wart virus in patients with different types of wart*

Precipitating antibody in serum taken when wart was		Type of wart					
Active	Regressing	SP	MP	H	F	G	Total
−	+	17	9	0	0	1*	27
+	+	2	3	0	0	0	5
+ (IgM)	+ (IgG)	6	2	0	0	0	8
(ND)	+	18	3	3	0	0	24
+	(ND)	8	5	1	0	0	14
−	(ND)	71	25	10	9	18	133
−	−	15	13	6	1	3	38
	Total	137	60	20	10	22	249
With precipitin (%)		37	37	20	0	5	31

SP = simple plantar; MP = mosaic plantar; H = hand; F = facial; G = genital. IgM, mercaptoethanol sensitive antibody: IgG, mercaptoethanol resistant. + = Precipitin present; − = precipitin absent. ND, serum not available.

* History of hand warts 4 years before.

Complement fixation tests

Rising titres of complement-fixing antibody to wart virus were demonstrated in paired sera from 20 out of 30 patients, 67 %. The wart virus antigen fixed complement in the presence of antibody, optimal concentrations of antigen being of the order of 10^9 virus particles/ml. Non-specific fixation with the control antigen (uninfected) was not seen. The complement-fixing antibody titres are compared with precipitating antibody in sera from cases with simple plantar warts showing significant changes in the titres of complement-fixing antibody over the period tested. Complement-fixing antibody was found only in people whose warts were regressing; it appeared later than precipitating antibody and disappeared sooner after cure of the lesion, as is illustrated by some of the cases in Table 2, and the rabbit sera in Table 3.

Table 3 shows the response in the rabbits immunized with wart virus antigen. It illustrates the features of the classical immune response, with the early appearance of 2-mercaptoethanol sensitive IgM which later is replaced by IgG. This was found also in the complement-fixing antibody response. In early sera complement-fixing activity appeared in the heavy fractions of the sucrose density gradients, in the position occupied by the Paul Bunnell IgM antibody, and in the later sera

in the fractions above the middle of the tube where IgG is expected. Complement fixation by IgM has been commonly found to be more efficient in the presence of increased concentrations of antigen (Pike, 1967). This could not be shown with wart virus as the higher concentrations were anti-complementary.

Table 2. *The development of complement-fixing and precipitating antibodies to wart virus in patients with warts*

	Complement-fixing and precipitating antibody in serum			
	Taken when the wart was			
Patient	Active	Regressing	Cured	Taken after stated period after cure
A. D.	< 8 (−)*	128	32 (+)	.
J. P.	< 8 (−)	.	64 (+)	.
C. W.	8 (−)	128 (+)	.	.
E. R.	< 8 (+)	32 (+)	16 (+)	.
V. L.	< 8 (−)	.	64 (+)	< 8 (+) 3 months
M. B.	< 8 (−)	16 (+)	.	< 8 (+) 1 year
A. M.	.	.	64 (+)	< 8 (+) 10 months
H. M.	.	.	32 (+)	< 8 (+) 14 months
A. D.	< 8 (−)	16 (+)	.	< 8 (+) 18 months
H. P.	.	32 (+)	.	< 8 (+) 2 years
S. M.	.	16 (+)	.	< 8 (+) 1 year

* CF antibody given as reciprocal of titre; precipitin result in parentheses.

Table 3. *Antibody response in rabbits after immunization with wart virus*

			Precipitating antibody in serum			
Rabbit no.	Time (weeks)	Inoculation*	Untreated	Treated with 2-ME	CF titre	Class of CF antibody
1	Preinoc.	.	−	−	< 8	.
	0	s.c.	.	.	.	.
	1	.	+	−	< 8	.
	2	.	+ +	+	128	IgM
	3	.	+ +	+ +	512	.
	10	i.v.	.	.	.	.
	11	.	+ +	+ +	512	IgM + IgG
	20	.	+	+	16	.
	24	.	+	+	32	IgG
2	Pre-inoc.	.	−	−	< 8	.
	0	i.v.	.	.	.	.
	1	i.v.	+ +	+	16	.
	2	i.v.	+ +	+ +	64	IgM
	3	.	+ +	+ +	512	.
	4	.	+ +	+ +	128	IgM + IgG
	10	i.v.	.	.	.	.
	11	.	+ +	+ +	512	.
	24	.	+	+	64	IgG

* 10^{11} Particles at each inoculation. s.c. = Subcutaneously with adjuvant; i.v. = intravenously. 2-ME = 2-mercaptoethanol.

This classical pattern of antibody development occurs in patients receiving treatment and in those who do not but whose warts undergo spontaneous regression. Few of the latter come to clinics, but sera were obtained from some who did and precipitating and complement-fixing antibodies were found in sera from four out of six, two with simple plantar warts and two with hand warts.

Passive haemagglutination tests

In view of the limitations imposed by the insensitivity of the precipitin test and the late development of complement-fixing antibody to wart virus, a more sensitive test was sought. An attempt to coat tanned red cells with the virus and to demonstrate agglutination in the presence of specific antibody was successful. This method proves to be very sensitive detector of antibody, particularly of IgM which is a much more efficient haemagglutinin than IgG (Osler, Mulligan & Rodriguez, 1966). This means that early high titres of this antibody were seen in some people whose sera were negative in other tests, and this was confirmed by the significant reduction in titre following treatment with 2-mercaptoethanol. Table 4 shows the results with sera tested by the passive haemagglutination and CF and precipitin tests and showing a significant titre of antibody by at least one of the methods, with examples from persons with each type of wart.

Table 4. *Comparison of antibodies detected by the passive haemagglutination test with precipitins and complement-fixing antibodies*

		Antibodies in serum taken when the wart was							
		Active				Regressing			
Patient	Type of wart	*PHA (U)	PHA (2-ME)	PT	CFT	PHA (U)	PHA (2-ME)	PT	CFT
R. L.	MP	320	10	−	< 8	640	320	+	8
L. B.	MP	160	40	−	< 8	640	320	+	< 8
C. M.	SP	20	ND	−	< 8	2560	640	+	32
M. G.	SP	10240	1280	−	< 8	2560	1280	+	ND
M. P.	SP	20	ND	−	< 8	320	320	+	< 8
B. B.	SP	640	< 10	−	< 8	160	20	−	32
S. S.	H	ND	ND	ND	ND	10240	ND	+	8
J. M.	H	ND	ND	ND	ND	160	ND	−	< 8
B. J.	H	2560	ND	+	< 8	ND	ND	ND	ND
W. C.	H	ND	ND	ND	ND	2560	ND	+	8
M. S.	F	ND	ND	ND	ND	320	ND	−	< 8
V2	G	640	ND	−	< 8	320	ND	−	< 8
V7	G	160	ND	−	< 8	ND	ND	ND	ND
V14	G	1280	ND	−	< 8	ND	ND	ND	ND

* PHA = passive haemagglutination titre in untreated serum (U) and after treatment with 2-mercaptoethanol (2-ME).

PT = precipitin result; CFT = reciprocal of complement fixation titre.

ND = not tested. For type of wart see Table 1.

Antigenic relationships

In all the serological tests, antigen from simple plantar warts was used as it was most abundantly available. When antigen from hand warts or mosaic plantar warts was substituted in the tests no variation in the results was found. The reaction of identity of these three antigens can be seen in Pl. 1 fig. 1. There were insufficient amounts of virus in the facial or genital wart material for use as antigen. However, the rabbit inoculated with genital wart material did develop antibody to wart virus, producing a precipitin that gave a reaction of identity with precipitins in sera from a patient with a simple plantar wart and a rabbit immunized with the virus (Pl. 1, fig. 2).

Specificity of the tests

The precipitin lines formed were in most cases single, curving towards the antigen well. Removal of the precipitate and examination of it by electron microscopy confirmed the belief that it indicated union of virus with antibody. Aggregates of viral particles were seen surrounded by a haze of antibody, or in some areas individual particles with an attached loop of antibody could be seen (Pl. 2, figs. 1, 2), features of antigen–antibody complexes already described by Almeida, Cinader & Howatson (1963). In some tests with human sera two lines could be seen. When these were extracted and examined separately similar aggregates were seen in both; there was no separation of full and empty particles as occurs with enteroviruses in precipitin tests (Conant & Barron, 1967). No particles were seen in agar from areas on either side of the precipitin line.

Complement fixation was also associated with the virus particles, the activity sedimenting with them on ultracentrifugation. No soluble complement-fixing antigens were detected.

Negative staining of washed, saponin-lysed sensitisized red cells as used in the passive haemagglutination test revealed the presence of virus particles on the cell membrane. Pre-incubation of sera with antigen (wart virus) completely inhibited the passive haemagglutination; other antigens (herpes simplex, adenovirus) did not.

Antibody in people not currently infected with warts

Sera from 34 girls who reported never having had warts were examined by precipitin and passive haemagglutination tests for antibody to wart virus. Of these, 25 had PHA titres of 40 or less (13 had 10 or less), 3 had titres of 80, 5 of 160 and one had 320. This last also had precipitating antibody and was the only one to do so.

Sera from a further 76 girls with a past history of warts were also examined. Six had precipitating antibody; three of these had had plantar warts within the previous 2 years and one within 5 years; the other two had had hand warts 3 and 7 years earlier. PHA titres of 80 and over were found in 7 out of 26 with a history of hand warts, and in 20 out of 50 with previous plantar warts. The two longest times since last known infection in these cases were 9 and 10 years.

DISCUSSION

The precipitin test does have the advantage of being easy to use, straightforward, and its results are very satisfactory when positive. However, its limited sensitivity is a drawback, especially in the study of an infection such as warts where the viral antigenic stimulus is small and often much of it is removed by the treatments employed, so that many patients can only develop low titres of antibody. Where the antigenic relation of viruses is being examined, precipitin tests are most valuable as any differences will show up in the formation of crossed lines or spurs or other evidence of reactions of non- or partial identity. The only difficulty lies in the accumulation of sufficient virus for such a test, and this may be easier in certain areas than others, depending on the treatment favoured for any type of lesion.

The significance of the occasionally observed second band of precipitation is not yet clear. It has been noted by others including Le Bouvier, Sussman & Crawford (1966), who found that, as was seen here, antigens composed entirely of full or empty particles gave identical lines, not separate ones. They found the second line formed with some old empty preparations. There were no cross-reactions among the papillomaviruses from man, dog, cattle or rabbits, or polyoma virus of mice.

Complement fixation by wart virus particles, and not by any soluble antigen extracted from the tissues, is confirmed by S. D. Elek (personal communication) and by Almeida, Oriel & Stannard (1969). In 1935 Maderna reported on a complement fixation test in which he used saline extracts of genital warts as antigen, finding some antibody in sera from 24 out of 45 people with this type of wart. It seems unlikely from present experience that the extract he used can have contained enough virus to give this reaction; possibly some other antigen may have been involved.

Complement fixation by IgM as well as IgG antibody is seen with wart virus antisera, and does occur in some other systems, as with herpes simplex virus, (I. W. S. Smith and J. F. Peutherer, (personal communication)), but not in all (Pike, 1967). Complement-fixing antibody is late in developing in several viral diseases, for instance rubella, and does tend to disappear sooner than other types so that its presence can be taken as an index of relatively recent infection. (Sever *et al.* 1965; Schmidt & Lennette, 1966). The results in this study with warts show the same pattern.

Passive haemagglutination is not a test commonly employed in virology as alternatives with adequate sensitivity are usually available, such as neutralization or haemagglutination inhibition tests. PHA has been applied to the study of herpes simplex virus (Scott, Felton & Barney, 1957) and to adenoviruses (Lefkowitz, Williams, Howard & Sigel, 1966). In both these investigations it was found necessary to use a slightly acid pH (6·4) for the sensitization of cells with virus, as noted in this study, and not the neutral pH used with some soluble protein antigens. This test has obvious advantages in detecting particularly IgM antibody to wart virus, and the lower levels of IgG not shown by other tests. However, more work requires to be done, for instance using purer antigen preparations, before the significance

of the titres obtained can be established. It is not altogether surprising that sera from nine out of the 34 girls who thought they had never had a wart did react with the virus in the PHA test. More than one person has been found on examination to have warts of which they were unaware.

The specific inhibition of PHA by pre-incubation with the antigen could be used to determine the smallest amount of virus that will do this, and the test used in this way for detecting virus. Reversed passive haemagglutination, where cells are coated with the antibody and antigen is measured, could also be very useful for this purpose. So far attempts to apply this method in the wart virus system have not been very successful, probably because of an insufficiently high titre of antibody in the sera used.

The question of the antigenic identity of viruses from all types of wart is at present confused. The viruses in what are termed skin warts (hand, feet and face warts) are probably of one type; certainly those from hand, simple and mosaic plantar warts appear identical. (Pl. 1, fig. 1). The evidence regarding the virus from genital warts is different. In this study, sera from people with genital warts (and no history of skin warts) reacted with virus from skin warts in the PHA test only. This was not considered surprising as all the individuals had small warts, and the paucity of virus in this type had already been observed (Dunn & Ogilvie, 1968). A recent report, however, describes the results of complement fixation and immune electron microscopy investigations of the relation of these viruses (Almeida *et al.* 1969). They found that although sera from patients with skin warts fixed complement with and agglutinated genital wart virus, sera from those with genital warts did this only with virus from genital warts, not with skin wart virus. A one-way antigenic cross between the two viruses was postulated. Serum from the rabbit inoculated with genital wart material in this project did react with skin wart virus, however (Pl. 1, fig. 2). Greater quantities of genital wart virus must be obtained before this question can be resolved.

The relation in time of the appearance of antiviral antibody to the regression of warts is interesting. It had not previously been shown that people whose warts underwent spontaneous resolution developed antibody. Indeed Epstein (1967) thought it unlikely that they did so. Antiviral antibody probably plays no part in the cure of warts, which must surely depend on cell-mediated immune responses, involving recognition of altered cell-surface antigens induced by the virus infection, as is seen in the regression of other viral tumours and homograft rejection. However, it is perhaps during the destruction of the infected cells that the virion antigens, normally rather inaccessible inside the cell, are exposed to the antigen recognition system and stimulate the appropriate antibody response. This antiviral antibody would then be of importance in conferring immunity to re-infection with this virus. Inadequate numbers of patients have as yet been followed for a sufficiently long time to allow determination of whether this is indeed so. Such a relationship between antibody and immunity, and not between antibody and tumour regression has recently been revealed in a study of bovine papilloma virus infection (Lee & Olson, 1969).

The author would like to acknowledge with thanks all the constructive interest shown in this project by Dr R. H. A. Swain and Dr Valerie Inglis. Mr R. Hopkins helped to carry out some of the work in establishing the passive haemagglutination test (Hopkins, 1969). The work could not have been undertaken without the support of Dr M. Bunney and Dr D. H. H. Robertson of the Royal Infirmary, Edinburgh, whose patients supplied the specimens. The author is also indebted to the Medical Faculty, Edinburgh University, for a Graduate Research Scholarship.

REFERENCES

ALMEIDA, J. D., CINADER, B. & HOWATSON, A. F. (1963). The structure of antigen–antibody complexes. A study by electron microscopy. *Journal of Experimental Medicine* **118**, 327–39.

ALMEIDA, J. D. & GOFFE, A. P. (1965). Antibody to wart virus in human sera demonstrated by electron microscopy and precipitin tests. *Lancet* ii, 1205–7.

ALMEIDA, J. D., HOWATSON, A. F. & WILLIAMS, M. G. (1962). Electron microscope study of human warts; sites of virus production and nature of the inclusion bodies. *Journal of Investigative Dermatology* **38**, 337–45.

ALMEIDA, J. D., ORIEL, J. D. & STANNARD, L. M. (1969). Characterisation of the virus found in human genital warts. *Microbios* **1**, 225–32.

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.

CARTER, R. L. (1966). Antibody formation in infectious mononucleosis. *British Journal of Haematology* **12**, 259–67.

CONANT, R. M. & BARRON, A. L. (1967). Enhanced diffusion of enterovirus antigens in agar gel in the presence of protamine. *Virology* **33**, 547–9.

CRUICKSHANK, R. (1965). *Medical Microbiology*, 11th edn. p. 913. Edinburgh: Livingstone.

DUNN, A. E. G. & OGILVIE, M. M. (1968). Intranuclear virus particles in human genital wart tissue: observations on the ultrastructure of the epidermal layer. *Journal of Ultrastructure Research* **22**, 282–95.

EPSTEIN, W. L. (1967). Cited by Rowson, K. E. K. & MAHY, B. W. J. (1967). Human papova (wart) virus. *Bacteriological Reviews* **31**, 110–31.

EVANS, C. A., GORMAN, L. R., ITO, Y. & WEISER, R. S. (1962). Anti-tumour immunity in the Shope papilloma-carcinoma. *Journal of the National Cancer Institute* **29**, 277–85.

FRITHIOF, L. & WERSALL, J. (1967). Virus-like particles in papillomas of the human oral cavity. *Archiv für die gesamte Virusforschung* **21**, 31–44.

GOFFE, A. P., ALMEIDA, J. D. & BROWN, F. (1966). Further information on the antibody response to wart virus. *Lancet* ii, 607–9.

HERBERT, W. J. (1967*a*, *b*). In *Handbook of Experimental Immunology*, Ed. D. M. Weir. (*a*) p. 702; (*b*) p. 1207. Oxford: Blackwell.

HOPKINS, R. (1969). B.Sc. Thesis, Bacteriology Department, Edinburgh University.

LE BOUVIER, G. L., SUSSMAN, M. & CRAWFORD, L. V. (1966). Antigenic diversity of mammalian papillomaviruses. *Journal of General Microbiology* **45**, 497–501.

LEE, K. P. & OLSON, C. (1969). Precipitin response of cattle to bovine papilloma virus. *Cancer Research* **29**, 1393–7.

LEFKOWITZ, S. S., WILLIAMS, J. A., HOWARD, B. E. & SIGEL, M. M. (1966). Adenovirus antibody measured by the passive haemagglutination test. *Journal of Bacteriology* **91**, 205–12.

MADERNA, C. (1935). La deviazione del complemento in infermi affetti da condilomi acuminati. *Riforma medica* **51**, 93–4.

MELNICK, J. L. (1962). Papova virus group. *Science, New York* **135**, 1128–30.

OSLER, A. G., MULLIGAN, J. J. & RODRIGUEZ, E. (1966). Weight estimates of rabbit anti-human serum albumin based on antigen-binding and precipitin analysis: specific haemagglutinating activities of 7S and 19S components. *Journal of Immunology* **96**, 334–44.

PIKE, R. M. (1967). Antibody heterogeneity and serological reactions. *Bacteriological Reviews* **31**, 157–74.

RUITER, M. & VAN MULLEM, P. (1966). Demonstration by electron microscopy of an intranuclear virus in epidermodysplasia verruciformis. *Journal of Investigative Dermatology* **47**, 247–52.

SCHMIDT, N. J. & LENNETTE, E. H. (1966). The complement-fixing antigen of rubella virus. *Proceedings of the Society of Experimental Biology and Medicine* **121**, 243–50.

SCOTT, L. V., FELTON, F. G. & BARNEY, J. A. (1957). Haemagglutination with herpes simplex virus. *Journal of Immunology* **78**, 211–13.

SEVER, J. L., HUEBNER, R. J., CASTELLANO, G. A., SARMA, P. S., FABIYI, A., SCHIFF, G. M. & CUSUMANO, C. L. (1965). Rubella complement-fixation test. *Science, New York* **148**, 385–7.

SMITH, J. W., PINKEL, D. & DABROWSKI, S. (1969). Detection of a small virus in a cultivated human Wilms' tumour. *Cancer (New York)* **24**, 527–31.

WATSON, D. H., RUSSELL, W. C. & WILDY, P. (1963). Electron microscopic particle counts on herpes virus, using the phosphotungstate negative staining technique. *Virology* **19**, 250–60.

EXPLANATION OF PLATES

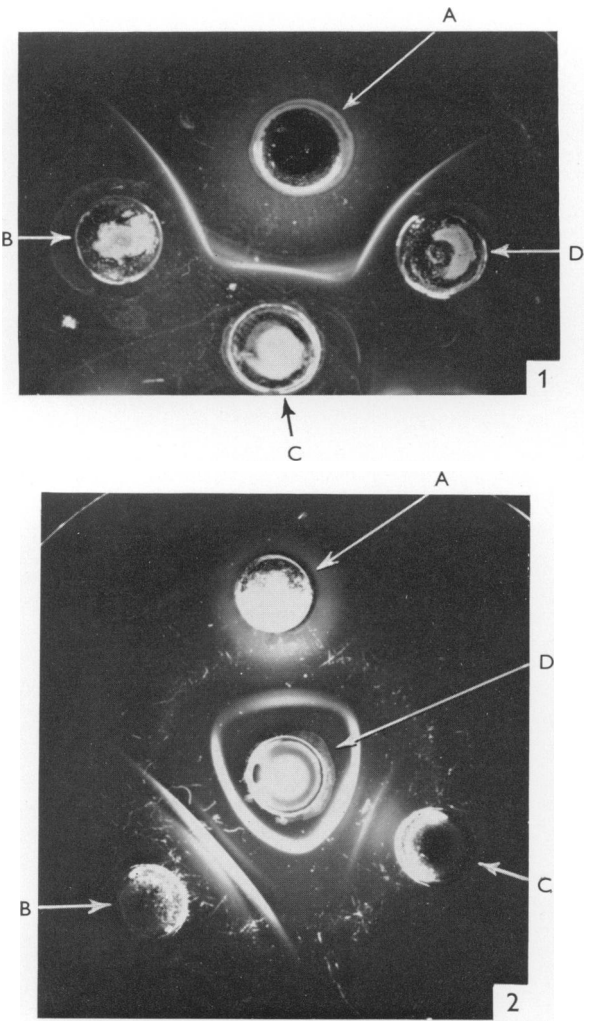
PLATE 1

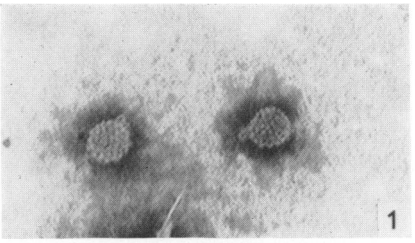
Fig. 1. Gel diffusion test record. Well A, serum from patient with simple plantar wart; well B, virus antigen from simple plantar warts; well C, virus antigen from mosaic plantar warts; well D, virus antigen from hand warts.

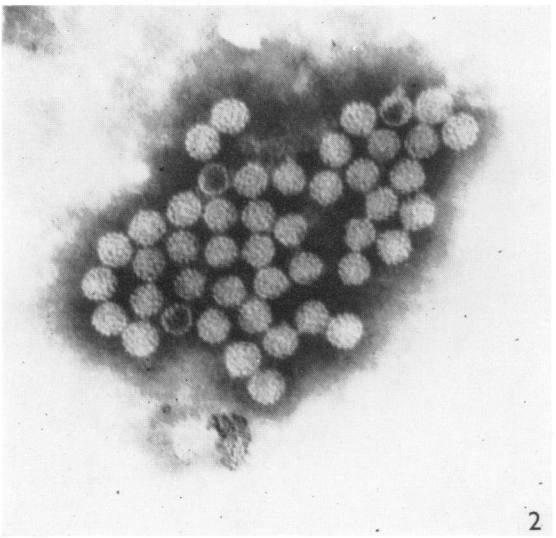
Fig. 2. Gel diffusion test record. Well A, serum from patient with simple plantar wart; well B, serum from rabbit inoculated with genital wart material; well C, serum from rabbit inoculated with virus antigen from simple plantar warts; well D, virus antigen from simple plantar warts.

PLATE 2

Figs. 1, 2. Negatively stained precipitate from gel-diffusion test, showing wart virus particles complexed with antibody. PTA stain. Pl. 2, fig. 1. × 120,000. Pl. 2, fig. 2. × 80,000.


A
B
D
C
1
A
D
B
C
2


1


2