THE DIAGNOSIS OF DISEASE RELATED TO THE HUMAN PAPILLOMA (WART) VIRUS.

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INTRODUCTION.

There is one form of tumour in man which has been clearly shown to be due to a virus. This is the wart, or papilloma, a circumscribed benign tumour occurring on epithelial surfaces. Warts are so common that the laity recognises them usually without difficulty. Clinically they have to be distinguished most often from epidermal lesions of different aetiology, such as senile seborrheic warts and the nodules of molluscum contagiosum. The latter condition is also caused by a virus, and as hyperplasia of cells is part of its histological picture it can be considered a tumour. However the lesions are very small (average diameter 2 mms) and there are no suggestions of malignant change occurring in them. Warts on the other hand can sometimes grow to a considerable size and do have certain significant associations with carcinoma in man and animals that will be discussed later.

The essential histologic feature of warts is a localised hyperplasia of cells in the upper layers of epithelium, the basal layer remaining unaltered. Various secondary changes in underlying tissue, the nature of the epithelium involved and environmental factors such as local pressure, friction or moisture, account for a wide variety of morphological appearances, which are described in detail in the text-books of pathology. Thus many names are used to denote warts as they appear in different sites on the body. Examples in general use at present are the common wart (verruca vulgaris), seen usually on the hands, and filiform or digitate warts (long thread-like or branching tumours) which are found mainly on the face or neck. Plantar warts are those on the soles of feet, plane warts are very smooth, flat warts seen particularly in young children, and condylomata acuminata are warts in the genital area. Dermatologists recognise two forms of plantar warts. The simple type is commonest in children, often extremely painful, and responds fairly rapidly to treatment. This type corresponds generally to dome-shaped "myrmecia" of Lyell...
and Miles (1951). The other, mosaic, type of wart is found usually in adults, is often not at all painful but is notoriously persistent, and may remain present in spite of treatment for two to three years or much longer. It takes its name from the characteristic "mosaic pavement" appearance of the lesion, several small, contiguous areas of skin being affected. This appearance is also sometimes seen in warts on the palms or fingers.

There are wart-like growths occurring on epithelial surfaces other than skin that share the basic histology of common warts. These include papillomata of the oral mucous membrane, laryngeal papillomata, ano-rectal warts, and perhaps bladder papillomata. The last-named occur on transitional epithelium, not on stratified squamous epithelium as the others do.

Common warts of man were shown to be transmissible from person to person many years ago. (Variot 1894, and Jadassohn 1896). In 1907 Ciuffo demonstrated that the infective agent could be passed in a bacteriologically sterile, cell free filtrate of warts, indicating that it might be a virus. Virus-like particles were first seen in warts by Strauss, Shaw, Bunting and Melnick (1949), who studied shadowed preparations of aqueous extracts of warts in the electron microscope. Electron microscopy of thin sections of warts revealed the virus-like particles in nuclei of cells. (Bunting, 1953). More recently it has been shown that the virus first appears in the nuclei of cells of the stratum spinosum in wart epidermis, and larger aggregates of virus are seen in cells above this layer, but no virus particles have been found in the basal layer of cells. (Almeida, Howatson and Williams, 1962). These authors also showed that large aggregates of the virus correspond to the basophilic intra-nuclear inclusions which are seen in stained sections of some warts on light microscope examination. (Lipschutz, 1924, and Blank, Buerk and Weidman 1951). Eosinophilic inclusions/
inclusions have been seen more frequently in warts (Strauss et al 1949, Lyell et al 1951); however they do not represent virus: it is thought they are connected with some abnormalities of Keratinisation found in warts and other conditions (Almeida et al 1962).

The virus of warts, human papilloma virus, is classified with similar papilloma viruses of other animals and certain other oncogenic viruses in the PAPOVA virus group (Melnick 1962). These are all small viruses (40–50 nm) containing double stranded deoxyribonucleic acid, replicating within the nuclei of cells, and able to produce tumours in certain hosts. They all show the same morphology on electron microscopy, having their capsomeres arranged with cubical symmetry, and are sometimes seen in filamentous forms. Much has been learnt about viral oncogenesis from studies of two members of the group, polyoma virus of mice and vacuolating virus of monkeys, SV 40. Unfortunately the papilloma viruses have not proved so easy to work with, and indeed so far the human papilloma virus has not been successfully propagated in any host other than man. (Many attempts have been made and occasional apparent success reported, but such results have never been repeated and confirmed). This major disadvantage has of course greatly restricted the scope of studies of this virus.

Human papilloma virus can be readily extracted from warts, and the fine structure of the virus has been studied in some detail in the electron microscope. Negative staining has revealed it as a nearly spherical virus with an outer coat (capsid) composed of projecting sub-units (capsomeres) which are arranged with cubical symmetry. There is disagreement as to the number of capsomeres present. Early workers considered that there were 42 capsomeres, (Williams, Howatson and Almeida 1961, Howatson and Crawford 1963) but more recent analysis by Klug and Finch (1965) suggests that 72 is the correct/
correct number. The size of the virus in negatively stained preparations averages 52 μm diameter. In thin sections the diameter is smaller as a result of shrinkage during fixation, dehydration and embedding.

Particles with the characteristic morphology of human papilloma virus have been demonstrated in common warts, plantar warts and plane warts. (Almeida et al 1962). They have also been shown recently in the nuclei of cells from lesions of the condition known as epidermodysplasia verruciformis, which has the appearance of a multitude of plane warts covering much of the body. (Ruiter and Van Mullen, 1966). Another recent publication reports the finding of papilloma virus-like particles in nuclei of cells from two papillomata of the oral cavity. (Frithiof and Wersall, 1967). Both these papers publish electron micrographs which reveal some of the structural detail typical of papilloma virus in sectioned tissue.

Reports of finding virus-like particles in genital warts have appeared. Zimmer, Bahnsch and Grimm (1964) describe particles in size range 20 - 80 μm seen mainly in the cytoplasm of cells. It seems uncertain that these were papilloma virus. However Melczer (1965) found spherical particles of approximately 40 - 50 μm, located mainly intra-nuclearly, and these may well have been virus, though no structural detail is seen in micrographs. Extracts of genital warts did result in common warts after inoculation into the skin (Waelsch 1918, Goldschmidt and Kligman 1958), as did extracts of laryngeal papillomata. (Ullman 1923, Ishikawa 1936). Bladder papillomata and ano-rectal warts do not appear to have been examined as regards virus content or trans-missibility. Young (1964) does mention that intranuclear inclusions were seen in ano-rectal warts.

It may/
It may be that malignant transformation of warts does very occasionally occur. Examples found in the literature describe the change to carcinoma in condylomata acuminata (genital warts), with well-documented clinical and pathological evidence. (Siegel 1962, Harmel-Tourneur and Kalis 1964). Also the condition of epidermodyplasia verruciformis already mentioned has a reputed tendency to early carcinomatous degeneration, thought it must be kept in mind that there is also an inherited defect of the skin in this disease. It is perhaps this defect which makes for a lowered resistance to wart virus. (Ruiter 1966). There is a verrucous carcinoma found in the oral cavity, a malignant tumour with otherwise the general features of a wart. The single laryngeal papilloma in an elderly patient is known to have a considerable tendency to carcinomatous change, and so are bladder papillomata. These associations, taken in conjunction with the known carcinogenic properties of those related viruses (polyoma, SV 40 and rabbit papilloma virus) which are usually found in mild, benign infections in their natural hosts, must be of some significance. They make it a matter of great interest to ascertain which diseases in man are caused by the human wart virus and whether this virus is ever responsible for malignant change in the cells.

It is not always easy to show that a virus is the cause of any disease. Its presence in the host may be demonstrated by direct detection of virus in host tissues or by propagating the virus in some other host by inoculation of material from the patient. The demonstration of the development of specific antibodies to the virus by the host during the period of illness and recovery also helps to relate the virus to the illness in time. In all investigations results must be interpreted with care. Passenger viruses having no relation to the disease in question are sometimes found in tissues. Particularly in electron microscopy of tissue sections it is often difficult to see/
to see sufficient detail of structure to identify a virus with confidence. In the
case of some virus-induced tumours in animals it has been found that recognisable
virus particles cannot be detected in the cells. This is the situation with the
rabbit papilloma virus, which is found in large amounts in the simple papillomata it
produces in its natural host, the cottontail rabbit. However in the papillomata
produced in the domestic rabbit whole virus particles are hardly ever detected, though
the infectious nucleic acid of the virus can be extracted from them. These
papillomata in the domestic rabbit do show carcinomatous change. In other
instances, particularly in tumours caused by polyoma or SV40 virus, certain "foot-
prints" are evident in the tumour tissue, showing that the viral gene has been active
in the cells. (Melnick and Rapp 1966). These foot-prints take the form of new
intranuclear (tumour) antigens and changes in the cell surface (transplantation)
antigens which are specific for the particular virus causing the tumour. Animals
bearing the tumours, or previously infected with the virus naturally, develop anti-
bodies to these various antigens and may be able to resist the formation of tumours.

The demonstration of human papilloma virus in some warts has already been noted.
Almeida and Goffe (1965) have described the detection of antibodies to this virus in
sera from patients with warts. They used an extract from warts as antigen, and
demonstrated antibody by precipitation in gel diffusion tests and also by direct
observation of the coating of virus particles by antibody, using the electron
microscope. They found antibody in 19 of the 42 sera examined. Further investiga-
tion of the sera revealed that 13 out of 18 were completely degraded by treatment
with 2-mercaptoethanol, indicating that the antibody was all of the 19S type (Goffe,
Almeida and Brown, 1966).

The present investigation into virological and immunological aspects of human
papilloma virus disease is far from complete. I am actively engaged in developing
further/
further the techniques about to be described, and enlarging the extent of the work as more material becomes available. All the serological tests and the electron microscopy, with the exception of the preparation of thin sections, have been carried out by myself.

**MATERIAL AND METHODS.**

The majority of wart specimens available consist of the thick layers of keratinised cells on the surface of plantar warts and some hand warts. These superficial layers are pared off before treatment is applied: such warts are very rarely excised wholly. Occasionally filiform warts on the face or neck are removed by curettage, and a few of these have been obtained. Some genital warts removed by curettage or excision were obtained, in some cases punch biopsies from the lesions. Each wart was collected in veronal buffered saline containing Penicillin and Streptomycin and stored at - 70°C.

**Electron microscope techniques.**

Each specimen was prepared individually for examination in the electron microscope. For routine use a slight modification of the negative staining method described by Chambers, Ito and Evans (1966) was adopted. A small piece of wart tissue was teased out in a drop of distilled water, then a coated specimen grid was applied to the fluid and a drop allowed to dry on to the grid. Next the grid was passed briefly through distilled water so that salts and any large debris was washed off, and then a drop of phosphotungstic acid was allowed to dry on to it. (PTA 2+ made just alkaline, pH7.2, with NaOH). This modification avoided the formation of crystals on the grids. Where viscous, cellular material was examined (usually filiform or genital warts), it was found that much clearer preparations were obtained by using the surface spreading technique of Parsons (1963). In this method a fine/
a fine needle was inserted into the wart surface: on withdrawal of the needle a few cells adhered to it and were floated off on to a drop of PTA, being spread and disrupted by the surface tension of the drop. A grid was then applied in the usual way. When dry, the grids were examined in an AEI EM 6 electron microscope.

A series of twelve genital warts was examined by thin sectioning in addition to negative staining techniques. A portion of each wart was fixed in osmium tetroxide, subsequently dehydrated in ethanol and embedded in Epon. Sections were cut on a Cambridge (Huxley) ultramicrotome, stained in uranyl acetate and occasionally lead citrate. This embedding and sectioning was carried out by A.E.G. Dunn, electron microscopist recently in this department, and the grids were examined in conjunction with him. A report of our findings is to be published shortly (Dunn and Ogilvie, 1968).

Antigen preparations.

When sufficient tissue from any one type of wart was accumulated, the pooled material was minced finely with scissors and ground with sterile sand. The sand and cellular debris was brought down by slow centrifugation and the supernatant fluid then subjected to 30,000 rpm for 90 minutes in a Spinco model L ultracentrifuge. A pellet was obtained and resuspended in a very small volume of distilled water. Electron microscopy of such preparations revealed masses of virus particles but no other recognisable matter, and these preparations constituted the antigens used in serological tests. The virus content was estimated by counting the particles with reference to a latex suspension of known concentration, using the loop drop method of Watson, Russell and Wildy (1963).

Serological methods.

Blood samples were collected from adult patients with warts, when possible at their initial visit to the clinic and later after treatment. After clotting occurred/
clotting occurred, the serum was separated and stored at -30°C. Antibody to the wart virus was detected by double diffusion tests in agar gel, precipitin lines appearing between the well containing the wart virus antigen and those containing serum with antibody, after incubation at 37°C for up to three days. (Almeida and Goffe, 1965). A micro-technique using wells of diameter 2 mm. cut in agar on glass slides was very useful in conserving materials and giving rapid results — precipitin lines within 12 - 16 hours. Ionagar was used, made up in phosphate buffered saline pH 7.2 with 0.08% sodium azide added. Initially agar was used at a concentration of 0.8%, but this was changed to 0.7% and all the sera were re-tested in agar at this concentration, as it has been found more sensitive (J.D. Almeida, personal communication), presumably allowing better diffusion.

It was found that the wart virus antigen preparations will fix complement in the presence of certain specific antisera (unpublished data). To demonstrate complement fixation the standard four-volume micro-technique commonly employed in virology was used. (Grist, Ross, Bell and Stott, 1966). The optimal dilution of antigen was determined by chequerboard titration, and a virus concentration of $10^9$ particles per ml. was found satisfactory for the test. The antigen was diluted to the required concentration in the veronal buffer used as a diluent in the complement fixation test.

Preparation of antisera in rabbits.

Virus antigen prepared as already described was used to immunise rabbits. Antigen was diluted in phosphate buffered saline to give a concentration of $10^8$ virus particles per ml. 1 ml. of this diluted antigen was emulsified in 1 ml. of oil-
:arlacel according to the method of Herbert (1967), to provide an adjuvant effect.

Rabbit No. 1. was given a sub-cutaneous injection of 2 ml. of the antigen-
:adjuvant preparation.

Rabbit No. 2/
Rabbit No.2. was given three intravenous injections, at weekly intervals, of 1 ml. of the saline antigen preparation.

Both animals were bled before the first inoculation and at weekly intervals for four weeks. They were again bled after booster intravenous injections of 1 ml. saline antigen had been given to each, 2 months after the first injection. Serum was collected and stored at -30°C.

Rabbit No.3. was inoculated intra-dermally in four sites on the flanks with 0.3 ml. of a preparation of genital wart tissue. The pooled warts had been minced finely, passed through a very fine mesh sieve, then suspended in a small volume of saline. A further injection of 0.7 ml. was given later, subcutaneously, when sufficient tissue was available.

It was ascertained that all the preparations were free from bacterial and fungal contamination before they were inoculated.

RESULTS.

Demonstration of virus by electron microscopy.

It is possible to detect virus particles easily and rapidly in negatively stained preparations provided sufficient numbers of particles are present. The characteristic morphology of human wart virus is clearly seen in such preparations, (far better than in tissue sections), and the virus is readily recognised at a magnification of 20,000 x. (compare Figs. 1 and 2).

Typical papilloma virus particles, usually in large aggregates, were seen in 92% of the simple plantar warts examined (34/37). In the mosaic type of plantar wart virus was also found, but not in such quantity or in the majority of specimens, -50% contained...
contained virus (9/18). Considerable amounts of virus were found in 60% of the hand warts examined (9/15) though some of these specimens were very small indeed. In filiform warts from the face and neck it was much more difficult to locate virus as preparations contained much cellular material. Even when the cell spreading technique was used typical papilloma virus particles were seen in only three out of thirteen specimens (23%).

It may be of significance that in two samples of parings from clinically cured plantar warts considerable numbers of virus particles were seen, but approximately half the number in each were penetrated by the PTA stain, appearing as empty shells. This indicates that the central core of DNA was missing and the particles were presumably non-infective.

Virus particles were not seen in any negatively stained preparations of genital warts which were examined individually, but a very few were seen in an antigen preparation made from the pooled warts. In the series of twelve genital warts examined after thin sectioning, papilloma virus-like particles were located in the nuclei of cells in the stratum granulosum of one wart. See Fig. 1. The size of the particles was 36 - 47 mu diameter, and the nuclei showed margination of chromatin. Particles of the same size but revealing no obvious structural details were seen in stratum granulosum cells of two other genital warts, and also in the negatively stained preparations made from these. These particles and those with the morphology of papilloma virus were seen to be closely associated with filamentous strands composed of regular sub-units. Such strands were first reported by Smith, Dougherty, Melnick and Rapp in common warts, and they suggested they could be unassembled viral sub-units. In this investigation such strands/
strands have also been noted, again closely associated with virus particles, in negatively stained preparations of a face wart and a mosaica plantar wart (See Fig. 2).

No virus has yet been found on negative staining of preparations of the first two specimens of bladder papilloma, just recently obtained. Specimens of oral papillomata have yet to be examined.

**Antigen Studies.**

Simple and mosaica plantar warts were the only types regularly available in adequate quantity to enable extraction of useful amounts of virus. A very small amount of virus antigen has just been prepared from the accumulated hand wart parings. All three antigens gave identical precipitin lines in gel diffusion tests against antibody-containing sera from patients with hand warts or simple or mosaica plantar warts. Thus the virus antigen appears to be common to the three types of wart examined in this way so far. All three antigens also fixed complement with the specific antisera. Pooled tissue from filiform warts was processed for the preparation of virus antigen, as was pooled genital wart tissue. In both these cases very few virus particles were seen on electron microscopic examination of the pellets, and the concentration was quite insufficient to produce precipitin lines or to fix complement. At least 10\(^{10}\) virus particles per ml of antigen was required before precipitation with antibody could be seen.

In order to ascertain that the precipitin lines were specifically due to wart virus-antibody reaction, the lines were cut out of the agar, stained with PTA/
PTA and examined in the electron microscope. Clumped masses of virus particles were seen with thin fibre-like structures joining them, presumably antibody molecules. In some cases there was a complete "fuzz" round the particles, and in others a single loop of antibody was seen attached to a particle. (See Figs. 3 and 4). All these appearances have been described by Almeida, Cinader and Howatson in studies of agglutination reactions between wart virus and antibody. Some sera gave more than one precipitin line (See Fig. 5) and these were examined separately in the electron microscope. No obvious difference between the lines was noted. Certainly there was not separate precipitation of whole and empty virus particles as is seen in the entero-viruses where there are group and type specific antigens.

Serological Studies

In sera from 87 patients with simple plantar warts, antibody to wart virus was detected by gel precipitation in 28 of the cases. In seven of these people, serum taken at their initial attendance at the clinic did not contain antibody but a later sample (six weeks or more) did. (Cases 4, 6, 8 and 9 in table). Also in two others the early sera gave very weak precipitin lines while later ones were much stronger. (Case 5). These 28 patients had had their warts for from two months to two and a half years at the time antibody was found in their blood. Four patients were found to possess antibody before they had received treatment of any kind. (Eg. Case 1).

Of nine patients with warts on their hands, only one possessed antibody (Case 14). None of seven people with warts on the face or neck had antibody. Only one of twenty-two patients with genital warts showed antibody. This was found in a second serum taken six months after biopsy of the lesion and three months after the patient was clinically considered cured. (Case 13). This patient /
<table>
<thead>
<tr>
<th>Case no. and serum</th>
<th>Prec. Ab.</th>
<th>Heat stable</th>
<th>CFT</th>
<th>Week type</th>
<th>up to time of sample treatment</th>
<th>clinical opinion of wart at time</th>
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</thead>
<tbody>
<tr>
<td>1. (AM) 1st</td>
<td>++</td>
<td>yes</td>
<td>64</td>
<td>SPW</td>
<td>2 months none</td>
<td>cured spontaneously</td>
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<tr>
<td>2. (MM) 1st</td>
<td>++</td>
<td>no</td>
<td>32</td>
<td>SPW</td>
<td>7 months podophyllin</td>
<td>cured</td>
</tr>
<tr>
<td>3. (HM) 1st</td>
<td>++</td>
<td>no</td>
<td>32</td>
<td>SPW</td>
<td>7 months podophyllin</td>
<td>cured</td>
</tr>
<tr>
<td>4. (JP) 1st</td>
<td>-</td>
<td>-</td>
<td>&lt;8</td>
<td>SPW</td>
<td>2 months none</td>
<td>active</td>
</tr>
<tr>
<td>5. (CN) 1st</td>
<td>++</td>
<td>yes</td>
<td>64</td>
<td>SPW</td>
<td>4 months podophyllin</td>
<td>disappearing</td>
</tr>
<tr>
<td>6. (VL) 1st</td>
<td>-</td>
<td>-</td>
<td>&lt;8</td>
<td>SPW</td>
<td>2 months none</td>
<td>active</td>
</tr>
<tr>
<td>7. (ER) 1st</td>
<td>+</td>
<td>no</td>
<td>32</td>
<td>SPW</td>
<td>8 months podophyllin</td>
<td>active disappearing</td>
</tr>
<tr>
<td>8. (EH) 1st</td>
<td>-</td>
<td>-</td>
<td>&lt;8</td>
<td>SPW</td>
<td>2 years + 3 months podophyllin</td>
<td>cured</td>
</tr>
<tr>
<td>9. (FF) 1st</td>
<td>+</td>
<td>no</td>
<td>60</td>
<td>SPW</td>
<td>2 years + 4 months podophyllin</td>
<td>active</td>
</tr>
<tr>
<td>10. (PC) 1st</td>
<td>++</td>
<td>yes</td>
<td>128</td>
<td>MPW</td>
<td>6 months podophyllin</td>
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</tr>
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<td>11. (SA) 1st</td>
<td>+</td>
<td>no</td>
<td>&lt;8</td>
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<td>8 months none</td>
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<tr>
<td>12. (VM) 1st</td>
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<td>active improving</td>
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<tr>
<td>13. (LI) 1st</td>
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<td>-</td>
<td>&lt;8</td>
<td>GW</td>
<td>8 months none</td>
<td>active</td>
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<tr>
<td>14. (BT) 1st</td>
<td>+</td>
<td>no</td>
<td>&lt;8</td>
<td>HW</td>
<td>3 years liquid N2</td>
<td>disappearing</td>
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Prec. Ab. = precipitating antibody found. 1 = very faint precipitin line
++ = very strong

CFT = reciprocal of titre of complement-fixing antibody

Week types: SP = simple planter  HP = hand wart
MP = mosaic planter   GW = genital wart
This patient was not known to have warts of any other type. Twenty-nine patients with mosaic plantar warts had their serum tested, and nine showed precipitating antibodies. Two of these people have continued to show weakly precipitating antibodies over a long period, with very little evidence of clinical improvement in their warts, and virus particles have been seen in the wart parings throughout this long period. (See Case 11).

Complement-fixing antibodies were detected in sera from twelve patients. In all cases the appearance of such antibody corresponded to the formation of strong precipitin lines and also to clinical cure of the wart within six weeks. It was possible to demonstrate rising titres of these CF antibodies where serial blood samples had been taken at the appropriate times. (Eg. Cases 4, 5, 6, 7). The titre of CF antibody fell within three months following cure of the wart in two cases (6,7).

Those sera which showed precipitating antibody were re-tested after exposure to heat at 65°C for one hour. Six continued to give precipitin lines after this treatment. All were from people who had developed CF titres of 1 in 64 or over and had been cured of their warts.

Rabbit Sera.

None of the rabbits showed antibody to the wart virus antigen in serum tested before immunisation. Rabbits 1 and 2 showed good responses to the antigen, producing precipitating and complement-fixing antibodies. The serum obtained in the first week from Rabbit 1 (which had received the antigen-adjuvant preparation) was the only one that had weakly precipitating antibody, heat labile and not fixing complement. Antibody in all the others was stable at/
at 65°C.

Rabbit No. 3. did not produce antibody to the wart virus antigen. Sera obtained after the booster dose of cell-suspension gave precipitin lines in tests against the wart virus antigen and the cell-suspension antigen. These represent non-specific anti-human antibodies, for they can be absorbed out with a suspension of normal stratum corneum cells. Thus so far no "tumour" antibodies have been detected.

DISCUSSION.

It is apparent that negative staining methods provide a simple and rapid means of demonstrating virus particles in warts. The results are particularly successful in those warts which have a thick layer of superficial keratinised cells, plantar warts and some hand warts, as these appear to contain the most virus. However, small amounts of virus will easily be missed with this relatively insensitive method, particularly if only a few cells are examined. The same criticism applies to the much more laborious procedure of preparing and examining thin sections of tissue.

But it is not often necessary to look for the virus, because clinical diagnosis of the lesions (by Dermatologists) is very accurate. Where demonstration of the virus can prove useful is in the differential diagnosis of an atypical lesion - is it a wart, or a simple corn, or another disease such as molluscum contagiosum? Recognition of the type of virus present in material from patients by electron microscopy is playing an increasing part in diagnostic virology. It is proving particularly useful in examination of cases of suspected smallpox and other skin lesions and in myxovirus infections of/
of the respiratory tract and meninges. The large pox-like virus of molluscum contagiosum is readily recognised. (See Fig.6). Until the wart virus can be propagated in tissue culture, electron microscopy of negatively stained specimens is likely to remain the method of choice for detecting its presence. For specific identification of course antigenic analysis is necessary.

It requires only patience to accumulate sufficient tissue before viral antigens can be prepared from all the different types of wart. It is satisfactory to be able to confirm that the virus in common hand warts and plantar warts, both simple and mosaic types, appears antigenically homogeneous. This has often been suspected from the clinical viewpoint as combinations of different types of wart may often be found in one patient, even occasionally the presence of both simple and mosaic plantar warts. What accounts for the typical appearance and persistent nature of mosaic warts is not yet clear. Perhaps a lack of local inflammatory response is indicated by the apparent spreading involvement of contiguous areas of skin. These mosaic warts are eventually cured.

The complement fixation technique is a very useful procedure, for detection of rising antibody titres helps to confirm the temporal relation of the virus to the disease process. In many acute viral infections the appearance of complement fixing antibodies corresponds to a recovery from the disease and development of a lasting immunity to it - as in the exanthemata. The complement fixing antibodies to wart virus found so far have all been in sera from patients who at that time were clinically considered to be cured or nearly so, and none have yet had any recurrence (up to one year from cure).
At the same time the antibodies in these sera were found to be heat stable (to 65°C for one hour), a characteristic of the lighter, 7S class of immunoglobulin, IgG (Pike, 1967). It has been shown in natural infections that the heat-labile 19S macroglobulin, IgM, is produced first and lasts only about ten days whereas the 7S type appears later and remains for a considerable time. If repeated, small antigenic stimuli are given, persistent 19S antibody production continues and protective immunity is not developed (Svehag and Mandels 1964).

Several sera from patients whose warts were cured did not contain antibody to the virus. It may well be that the antigenic stimulus was so small that no antibody response was elicited or only 19S antibody produced. As patients attend the clinic at intervals of six weeks, and often fail to report if their wart is cured in the interim, the transient appearance of 19S antibody could easily be missed. The complement fixing and heat stable antibodies were found only in patients with plantar warts, and all but one of these was of the simple type. It is to be noted that all the other types of wart contain much less virus than the simple plantar ones, and these other types recur more frequently after treatment.

It may be that humoral antibody to wart virus is not in itself responsible for eradication of the disease. Antibodies against the virus-infected cell may be of greater importance. Such tumour antibodies have not so far been detected in sera from humans with warts, or rabbits immunised with preparations of wart-infected cells (unpublished data), using extracts of wart tissue as antigens. This aspect of the investigation has to be carried a great deal further. Useful results may well not be forthcoming until the virus can be made/
made to reproduce in some tissue culture system (attempts to achieve this are also in hand), when tumour antigens should be detectable in infected cells. Once these "footprints" of wart virus activity can be identified in vitro, actual tumours can be examined for these antigens, probably using specific antisera in complement fixation techniques or indirect fluorescent antibody tests.

SUMMARY.

The human papilloma virus is known to cause certain benign lesions in man, and is suspected as an aetiological factor in some other tumours where occasional carcinomatous change is known to occur. Investigations into this subject have been limited because no means of propagating the virus outside its natural host are yet available.

Techniques for demonstrating the virus in tissues, and results of the examination of different types of wart are discussed. The demonstration of antibodies to the virus by precipitation in gel diffusion tests and by complement fixation is described. Some features of these antibodies, and also of antigens prepared from different types of wart are noted. The nature of further studies, directed particularly towards identifying virus-induced tumour antigens is indicated.
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MELNICK /


THIN SECTION OF A GENITAL WART

PAPILLOMA VIRUS-LIKE PARTICLES IN NUCLEUS OF CELL IN STRATUM GRANULOSUM.
TISSUE FROM MosaIC PLANTAR WART

NEGATIVELY STAINED. SHOWS TWO PAPILLOMA VIRUS PARTICLES AND ALSO STRANDS WITH A REGULAR SUB-STRUCTURE.
fig 3

PRECIPITIN LINE EXTRACTED FROM AGAR.  
NEGATIVELY STAINED. SHOWS PAPILLOMA VIRUS PARTICLES CLUMPED AND SURROUNDED BY "FUZZ" OF ANTIBODY MOLECULES.

fig 4

SHOWS PRECIPITIN LINES BETWEEN CENTRAL WELL CONTAINING ANTIGEN AND PERIPHERAL WELLS CONTAINING PRECIPITIN LINE EXTRACTED FROM AGAR.

NEGATIVELY STAINED. SHOWS TWO PAPILLOMA VIRUS PARTICLES EACH WITH AN ATTACHED "LOOP" OF ANTIBODY.
GEL DIFFUSION TEST PLATE
SHOWS PRECIPITIN LINES BETWEEN CENTRAL WELL (CONTAINING ANTIGEN) AND PERIPHERAL WELLS (CONTAINING ANTISERUM). NOTE TWO PRECIPITIN LINES SEEN WITH SERUM ON LEFT.
TISSUE FROM LESION OF MOLLUSCUM CONTAGIOSUM \( \times 100,000 \)

NEGATIVELY STAINED. SHOWS STRUCTURE OF THE VIRUS PARTICLE.