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VERRUCAE VULGARES

II. Demonstration of a Complement Fixation Reaction

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Abstract. A complement fixation reaction on the basis of antigen prepared from Verruca vulgaris provides an opportunity to demonstrate antibody in serum in a total of 20% of patients with warts. The quantity of antibody is seen to rise upon removal of the warts. In a group of blood donors who served as controls, such complement fixing antibodies were found in about 3% of the latter, the percentage being commensurate with the incidence of warts in the population at large.

Nothing much is known about the antibody response in cases of wart virus infection in man. According to all available reports on experimental transmission, only a small proportion of the subjects exposed have been found to develop warts, thus suggesting either that the virulence of the virus is low or that resistance on the part of the host is high. Human subjects are susceptible to reinfection even though they may have been completely cured and all warts, even the multiple ones, have disappeared within a few days. Regression may occur spontaneously or it may be induced, for instance by vaccine which, in the hands of some investigators, is a very efficient therapy (4). According to several investigators (Table II), the formation of antibodies may in some way be related to the occurrence of warts. So far, evidence in support of this postulation is not convincing and furthermore, the occurrence of warts and formation of antibodies in patients has not yet been followed over sufficiently long periods of time.

Although the tumour is readily accessible and the causative virus may be isolated in a relatively pure condition, propagation *in vitro* of wart tissue and wart virus has not been possible. This fact has proved a severe handicap in the effective study of this type of tumour.

The present paper is concerned with a complement fixation (CF) test involving wart virus antigen from warts and sera derived from patients. The results obtained by this test can, to a certain extent, be correlated with the clinical condition and the course of the infection.

MATERIAL AND METHODS

Sera from patients

Blood samples were allowed to clot at room temperature for 3–4 hours after which they were cleared by centrifugation on the same day. The sera, without addition of preservatives, were stored in the refrigerator at −20°C. Even though an aseptic technique was used during preparation and manipulation of these, some sera became contaminated with bacteria and were discarded. The remaining specimens were frozen and thawed several times, but still retained their titre. No anticomplementary activity developed. They were stored for at least 30 months. Prior to use they were diluted 1 : 4 in Veronal-buffered saline (VBS) and inactivated at +56°C for 30 min. Whenever possible, the serum samples were obtained from the patients immediately before treatment and, subsequently, at intervals of 1 week, 1 month, and 3–4 months.

Sera from donors

Serum samples were obtained at random among blood donors. The samples were treated as described in the above paragraph: Sera from patients.

Biopsies

Typical common warts (verrucae vulgares) localized to the hand or sole were used for the preparation of the CF-antigen. Warts presenting the following characteristics were preferred: Untreated warts with a diameter not greater than 3–4 mm and a history not exceeding 2 years; owing to the scarcity of warts of this type it might not

Table I. *Preparation and purification of wart antigen*

1.	Warts stored at −20°C for periods of time not exceeding 6 months.
2.	Pooling of about 25 biopsies, total weight about 2 grams
3.	1st homogenization: Ultra Turrax, 2 min
4.	Freezing in dry ice, chilling in water at +25°C (twice repeated)
5.	2nd homogenization: Potter–Elvehjem, 5 min
6.	1st centrifugation: 3 000 r.p.m. for 15 min (4 000 g) (supernatant)
7.	2nd centrifugation: 40 000 r.p.m. for 120 min (sediment)
8.	1st filtration: Sandwich of: Glass-filter 1.2 micron filter-pore and 0.4 micron filter-pore
9.	2nd filtration: 0.2 micron limiting pore-dimension
10.	Storage of filtrate=antigen at −70°C

always be possible to have these criteria fulfilled. Avoiding bleeding as far as possible, the warts were curetted under local anaesthesia, care being taken that the applied anaesthetic did not penetrate directly into the tumour (9). Prior to storage the biopsies were wiped clean of blood. They were frozen immediately in dry ice at −70°C and kept frozen until use. By the time the biopsies were used for antigen preparation, none had been stored for periods of time exceeding 6 months.

Preparation of CF-antigen

Antigen was prepared in the form of extracts from tumour tissue obtained under observance of aseptic precautions; as much as possible of the surrounding skin was removed and the specimens were stored in dry condition for varying periods. Subsequently the tissues were thawed and weighed, upon which they were washed no less than five times in 0.9% saline with phosphate buffer (0.1 M, pH 7.2) in a volume 100 times their weight.

Homogenization technique

(1) The tissue suspension was homogenized in an Ultra-Turrax (type 18/2, Janke – Kunkel) three times at 20 000 r.p.m. for 20 sec, followed by six times for 10 sec. Intervals of 30 sec between each homogenization procedure were sufficient for the appropriate cooling (in melting ice cubes). Upon homogenization, the milky fluid was frozen and thawed twice in dry ice/water at +25°C.

(2) The tissue was homogenized a second time in a Potter-Elvehjem homogenizer (Braun-Melsungen, Teflon pestle) and treated for 5 min at varying velocities. Care was taken to have the sediment at the bottom of the tube ground and suspended.

(3) Ultrasonication treatment (20 kcs/sec, 60 W), for 15 sec, of the homogenate mixed in equal parts with 100 micron glass beads completed the mechanical homogenization.

Centrifugation technique

(1) The tissue suspension was decanted, leaving the glass beads and major cell debris; the supernatant was centrifuged in a Sorvall RC 2-B automatic superspeed refrigerated centrifuge at +4°C at 3 000 r.p.m. for 5 min (3 000 r.p.m. equalling 4 000 *g*).

(2) The harvested supernatant liquid was subsequently centrifuged in a Spinco preparative ultracentrifuge (Model L Beckmann, Swinging Bucket Rotor, type SW 50 L) at 40 000 r.p.m. for 120 min at +4°C; the sediment, which was resuspended in saline, constituted the antigen to be used for special experiments. The supernatant from the 1st centrifugation was generally used for the preparation of the CF-antigen, ultracentrifugation being omitted because of the heavy loss of antigen involved in this procedure. The supernatant was a clear, slightly opalescent liquid which, microscopically, appeared homogenous and free from gross particles. It was filtered through a Millipore filter-sandwich and sterilized through an 0.2 micron filter. This filtrate constituted the antigen proper. It was distributed into 1.0 ml vials and stored at −70°C for 6 months during which period deterioratation did not occur according to measurement of its complement fixing activity. Inactivation at +56°C for 30 min prior to use did not produce any difference in the results and consequently it was omitted from the routine. Upon inactivation, the opalescence of the antigen was slightly intensified. The antigen was used immediately after thawing of the ampoule in a water bath. It should be noted, however, that the potency remained unaltered after incubation at +4°C for 2–3 days in the refrigerator. The antigen titre was determined, using rabbit hyperimmune serum as standard. The antigen unit equalled 0.1 ml of such dilution of the antigen as gave the maximal serum titre. Two antigen units per 0.1 ml were used in the test.

Salt solutions

(1) 0.9% NaCl; (2) 0.9% saline in phosphate buffer M/200, pH 7.2; (3) The Dulbecco Veronal-buffered saline (VBS) was used in the CF-test.

Complement

Fresh guinea pig serum obtained by heart puncture from 50–60 animals was pooled an stored in 1 ml vials at −70°C. The complement titre in each batch of antigen was determined immediately before use. Two antigen units

Table II. *Synopsis of studies of antibody formation in patients with warts*

Author	Year	Type of antibody	Lesion	Ref. no.
Maderna	1935	Complement fixing	Condyloma	14
Beard & Kidd	1936	Neutralizing	Verrucae	3
Brain	1937	None	—	7
Biberstein	1944	None	—	4
Bivins	1953	None	—	5
Fischer	1953	None	—	8
Klapötke & Langhoff	1953	None	—	12
Almeida	1965	Precipitating	—	2
Goffe	1966	Precipitating	—	11
Alexander	1966	Complement fixing	—	1

of complement per 0.1 ml were used in the actual test. The applied antigen dilution (1 : 4 or 1 : 8) would usually provide complement titres in the range between 1 : 40 and 1 : 60.

Haemolytic system

A 2% suspension of sheep erythrocytes in VBS (previously washed thrice in VBS) was made up for each experiment and mixed with equal parts of amboceptor (rabbit antibody to sheep erythrocytes) diluted to contain 2 haemolytic units per 0.1 ml.

CF-technique

Perspex plates were used throughout the investigation together with standardized, automatic pipette syringes. Serum samples were diluted two-fold in VBS, directly in the plates. 0.1 ml of antigen, serum, and complement were incubated overnight at +4°C. The haemolytic system was added the following day after incubation of the plates at +37°C for 1 hour. The plates were shaken four times during 1 hour and read 2 hours later. They were finally read on the following day after incubation for a further 18 hours at +4°C. This second reading was recorded as the actual end point. The highest dilution giving 75% complement fixation was taken as the serum titre. Controls for non-specific anti-complementary activity of antigen/serum were included every day together with negative and positive serum samples with known titres.

Control antigens

Antigen controls included: normal epidermis, hyperkeratosis, and basal cell skin carcinomas prepared in accordance with the previously described procedure used for the antigen proper. Material from bovine papillomatosis, molluscum contagiosum, and condyloma acuminatum was also included as virus control.

Positive hyperimmune serum

Rabbit antiserum obtained as previously described (10), was used as reference throughout the experiment.

Table III. *Series of examined patients*

The patients were classified into two groups according to development or non-development of CF-antibody to wart virus

	Men		Women		Total	
	No.	%	No.	%	No.	%
Presence of CF-antibody to wart virus[a]	23	22.5	14	15.2	37	19.1
Absence of CF-antibody to wart virus	79	78.5	78	84.8	157	80.9
Total	102	100	92	100	194	100

[a] Patients who at least once during the period of observation presented with positive CF-titre against wart virus.

Table IV. *Number of blood samples obtained from patients with warts*

The samples are classified into two groups: (1) samples obtained from patients in whom antibodies were present at least once during the period of observation and (2) samples obtained from patients in whom antibodies were absent

	Number of examined blood samples[a]		
	Presence of CF-antibody to wart virus	Absence of CF-antibody to wart virus	Total
Presence of CF-antibody to wart virus[b]	52 (14.3)	43 (11.9)	95 (26.2)
Absence of CF-antibody to wart virus		268 (73.8)	268 (73.8)
Total	52	311	363 (100)

[a] Figures with in parentheses are percentages of total.
[b] Patients who at least once during the period of observation presented with positive CF-titre against wart virus.

RESULTS

A procedure involving extraction by saline made it possible to produce an antigen from wart tissue; the antigen was free from unspecific and anti-complementary action and its potency was sufficient to have a serviceable standard antiserum prepared by repeated immunization of rabbits. The antigen and antiserum thus obtained is easily quantitated in a specific complement fixation reaction. The procedure is fully reproducible if only its course is followed closely during preparation of the antigen and in the choice of the required biopsy material; among about 40 antigen preparations, the five presenting the highest antigen titres were used in the present study, i.e. one unit determined as the dilution 1 : 32.

As controls, antigen preparations served which were produced from epidermal tissue of a structure resembling as far as possible that of the warts. A total of 11 epidermal antigen preparations and three hyperkeratosis antigen preparations were used. There were no cross reactions either between wart virus antigen and control standard sera or between control antigens and wart virus standard serum.

During the performance of the CF-test, the wart virus antigen was used in the dilution previously determined by antigen titration (2 antigen units per 0.1 ml); as it proved impossible to have the

Table V. *Number of serum samples from blood donors examined with a view to presence or absence of CF-antibodies to wart virus*

The number of serum samples equal sthe number of donors

	Men		Women		Total	
	No.	%	No.	%	No.	%
Presence of CF-antibody in serum	9	2.7	4	5.0	13	3.2
Absence of CF-antibody in serum	321	97.3	76	95.0	397	96.8
Total	330	100	80	100	410	100

equivalent optimal dilution of control antigens determined, the latter were used in various dilutions which all provided identical results. Hence, a fixed dilution of 1 : 128 was chosen; at this dilution, the w/v percentage of the saline extract would equal that of the wart virus antigen, calculated on the basis of the weight of the curetted tissue specimen.

In a series of 194 patients, including 92 (47.4 %) women and 102 (52.6 %) men, a total of 363 blood samples were obtained (Tables III and IV). In the selection of patients, the exclusive prerequisite has been that curettage and coincident removal of two or three warts would be practicable. All of the patients received this therapy and all warts were treated.

It appears from Table III that 37 out of 194 (19.1 %) patients at some time during their infection harboured antibodies of the type concerned; the formation of antibodies was found to be more common in men than in women (23/14), but the difference is not significant. A total of 95 blood samples were drawn from the 37 patients; antibody was in evidence in 52 of these samples (i.e. in 52 out of 363 = 14.3 % in the entire series); antibody was absent in 43 samples obtained in the same group of patients. Antibody to wart virus was absent in the remaining 268 samples derived from 157 patients. The distribution of samples obtained on the 1st, 2nd, 3rd, and 4th occasions is illustrated in Table VI. The development of titres is characteristic and is seen to change during the course of the infection. Antibodies were in evidence at early or late stages of the infection in 19.1 % of the patients with warts whereas only 12 out of 194 (6.2 %) proved to be sero-positive at the first consultation. The variations in antibody production are illustrated in Tables VI and VII from which it also appears that the very high titre values (above 1 : 128) were never observed among samples obtained early in the course (1st and 2nd specimens), but one of the maximal titre values was found among samples obtained late (in the group of 4th specimens). It appears also from Table VI that high positive CF-values were demonstrable only among samples obtained at late stages (2nd and 3rd specimens). A total of 67 examinations were performed in which the course of infection was followed via 3 or 4 blood samples. The rise in titre appears from Table VIII in which the percentage of positive and negative samples is calculated, the calculation applying to the entire series and to all intervals of time. The increase in the number of positive samples is seen to be gradual, a feature which is particularly marked in the group of 3rd samples.

In the control series (Table V) comprising 410 blood donors from whom 410 blood samples were

Table VI. *Distribution of CF-titre over 95 samples of serum*[a]

		CF-titre in the presence of wart antigen										
Sample drawn	Serial no.	Negative	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	Total positive	Positive + negative
Prior to treatment	1	25	2	3	1	4	1	1			12	37
After intervals of												
1 week	2	15	4	3	1	2	2	2	3	1	18	33
1 month	3	3	1	2	5	1	4	5	1		19	22
3 months	4	0				1		1		1	3	3
Total		43	7	8	7	8	7	9	4	2	52	95

[a] From 37 patients who at least once during the period of observation presented with a positive titre.

Table VII. *The relation between treatment and formation of antibody*

Number of patients in whom positive CF-titre against wart virus was present/absent

I	1° sample[a]	Positive	Subsequent	Negative	9
II	1°	Negative	samples	Positive	25
III	1°	Positive		Positive	3
Total number of patients in whom antibody developed					37

[a] Obtained prior to treatment.

obtained, reactions suggesting a presence of antibody (titre as low as 1 : 8) were seen in 13 cases (3.2 %). The distribution according to age of patients and donors included in the series is illustrated in Figs. 1 and 2; none of the donors was less than 18 years old. The age of patients averaged 18.1 years; that of the donors averaged 35.1 years. The distribution according to presence of antibodies in patients is illustrated in Fig. 3. All age groups were found to include subjects in whom antibody to wart virus was in evidence. All of the examined blood samples were subjected to control using saline; the few (3) unspecific reactions have been included as negative samples; the latter have not affected the statistical analysis of the results because they could be included as negative samples. In the presence of wart virus antigen, the serum titre 1 : 4 was encountered in 7 out of 95 samples (7.4 %) obtained from patients with warts; on the basis of this analysis it seemed justified to include such faintly positive reactions. All of the 363 sera were examined by means of control antigens produced from epidermis and hyperkeratosis according to the methodology used also in the case of wart virus antigen. The results are recorded in Table XI in which patients are classified according to the criterion: Positive or negative titre in the presence of wart virus antigen. Positive reactions with control antigens were seen only in the group of patients in whom antibody to wart virus was in evidence, but never in patients in whom it was absent. The potency of the recorded reactions was in all cases 1 : 8. Furthermore, in the group of patients with positive sera, all 95 specimens were examined by means of certain antigens produced from epidermal tissue, using the routine CF-test-methodology (antigen from molluscum contagiosum, condyloma acuminatum, basal cell carcinomas and bovine papillomas). Among the sera which reacted with the control antigens used in the routine (epidermal and hyperkeratosis antigens), three sera were found to react also with various other antigens (Table X); the order of magnitude of titres thus produced deviated essentially from the specific titres encountered in patients harbouring wart virus antigen (1 : 8 in all cases).

DISCUSSION

Studies, which otherwise might be instructive, are not available in the literature on the formation of antibody in man infected with wart virus. Consequently, series of patients to be studied must fulfil the sole requirement that surgical treatment of the individual patients include the coincident elimination of all wart tissue. Such radical removal of all tumour tissue is presumed to provoke essential changes in the immunological response of the

Table VIII. *Change of CF-titre during treatment of the warts*[a]

		Number of serum samples				
			After intervals			
	CF-titre	Prior to treatment	1 week	1 month	3 months	Total
Presence of CF-antibody to wart virus[b]	Negative	25 (14.0)	15 (12.8)	3 (5.9)		43 (11.9)
	Positive	12 (6.7)	18 (15.4)	19 (32.4)	3 (37.5)	52 (14.3)
Absence of CF-antibody to wart virus	Negative	142 (79.3)	84 (71.8)	37 (62.7)	5 (62.5)	268 (73.8)
Total		179	117	59	8	363

[a] Figures within parentheses are percentages of total.
[b] Patients who at least once during the period of observation presented with positive CF-titre against wart virus.

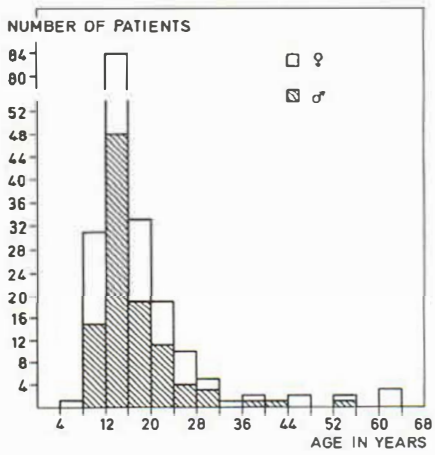

Fig. 1. Graphical representation of distribution according to age of patients with warts.

organism to the tumour. The formation of antibodies is demonstrable in serum after the tumour has been removed. The explanation of this formation may be two-fold: either virus is introduced into the organism during the operation, i.e. a genuine "vaccination", or a weak, although continuous, formation of antibody may for a while "overshoot the mark" until its stimulus disappears. Whether one or the other explanation is correct cannot be decided on the basis of the data obtained in the present study.

The viral aetiology (2, 3, 6, 13) is incontestable. Hence it seemed reasonable to presume that this

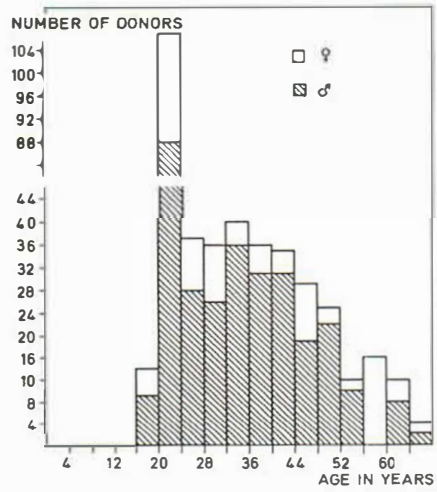

Fig. 2. Graphical representation of distribution according to age of donors.

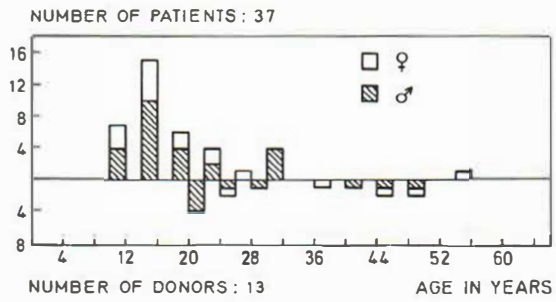

Fig. 3. Graphical representation of distribution according to age of CF-positive patients.

virus antigen might be usable in a serological reaction. In the preparation of the virus, a generally accepted technique was used (2, 6, 11, 17); it was modified accordingly as the established CF-technique permitted a specific determination of the antigen. Keeping strictly to the routine, it was possible to obtain antigen preparations with a high specific activity (CF-titre) which was reproducible from preparation to preparation. For technical reasons, the complement fixation reaction was preferred because of its specificity and sensitivity. The applied dilutions of antigen and of rabbit standard serum facilitated the establishment of a usable complement fixation reaction; the conclusive evidence of its specificity is that comparable antibodies are in evidence in sera from patients.

Spontaneous recovery within 4 years is to be ex-

Table IX. *Positive reactions in patients exposed to CF-tests in which extracts from epidermis and hyperkeratosis served as antigen*[a]

		Number of exposed patients	Blood samples obtained from the same patients
Presence of CF-antibody wart virus[b]	Positive	2	3
	Negative	35	92
	Total	37	95
Absence of CF-antibody to wart virus	Positive	0	0
	Negative	157	268
	Total	194	363

[a] The 3 positive sera were observed among the 52 samples of CF-positive sera derived from 37 patients.
[b] Patients who at least once during the period of observation presented with positive CF-titre against wart virus.

Table X. *Number of serum samples presenting with CF-antibody against control antigens*

Sample drawn	Serial no. of sample	Epidermis	Hyper-keratosis	Molloscum contagiosum	Condyloma acuminatum	Basal cell carcinoma	Bovine papilloma	Total
Prior to treatment	1		1					1
After intervals of								
1 week	2	1	1			1	1	4
1 month	3	1	1		1	1		4
3 months	4							
Total		2	3	0	1	2	1	9

CF-test of 95 sera from patients who at least once during the period of observation presented with a positive CF-titre against wart virus. Specification of control antigens. Using these antigens, titres were in all cases recorded as: 1:8.

pected in 90% of patients with warts (15); patients who have recovered from the infection may be susceptible to later re-infection. Neither the potency nor the duration of the immunity is yet known. If the formation of antibody were correlated with the course of infection and occurred in a minor group of patients, the reason might be either that concentrations of antibodies in serum were low or their presence there of short duration. In the present series, only 5% of the patients were found to harbour complement fixing antibodies before treatment was commenced, while a formation of antibody at later stages was observed in 20% of the patients, although the latter feature was seen only during, or rather after, treatment.

Since these antibodies are produced in only a few of the patients, it looks as though there might be an interplay of several factors. The generally small volume of antigen in warts accumulates close to the surface of these and occurs primarily in the nucleus, later in the cytoplasm; it is always separated from the organism and its antibody-producing cells by the basal membrane below the epidermis (10, 17). Furthermore, it is a well-known fact that the capacity on the part of papilloma virus to induce antibody in the host may vary (3, 6) and also that papilloma virus antigens are of particularly poor quality in the normal biological host unless infection is contracted via routes other than the normal ones. Among the treated patients, recovery will include all warts (in analogy with spontaneous healing) and, although it fails to protect against infections to be contracted later in life, this suggests in this connection that the formation of antibody may be correlated with the course of infection, probably even be directed against affiliated or partial antigens. It is remarkable that complement fixing antibodies reacting with control antigens are not in evidence in patients in whom antibody to wart virus is absent and the same applies to donors. Such negative reaction with control antigens has previously been described in a study concerning condyloma acuminatum (14).

The applied control antigens are not directly comparable with the wart virus antigen. The deviation is of a biochemical as well as morphological nature and thus, an application of these as control antigens will merely serve to exclude technical errors or indicate that reactions of certain sera with certain antigens are unspecific in the system concerned. For this reason it seems justified to anticipate the negative results. The explanation why no more than three sera presented with unspecific reactions may be that the interval between drawing of blood samples and their storage in a refrigerator covered only a few hours. So far, it has not been possible to measure the difference in potency of control antigens and virus antigens; neither are the titre values of sera titrated against the various control antigens fully comparable. It

Table XI. *Evaluation of serum samples with positive CF-titres against wart antigens and control antigens*

No. of serum sample[a]	71	95	99
Titre against wart antigen	1:256	1:128	1:128
Titre against control antigen	1:8	1:8	1:8

[a] All three sera reacted with hyperkeratosis antigen.

should be borne in mind, however, that it is a matter of a virus antigen, not of an epidermal antigen.

It is generally admitted, and confirmed also in the present study, that there is no antigenic relationship between human and bovine papilloma virus (6) (Table IX). Warts and condyloma acuminatum are generally presumed to be provoked by one and the same virus; in the present study, serum from one patient with warts was found to give a faintly positive reaction with condyloma antigen. Using the above-mentioned technique and wart virus antigen, 160 sera from 40 patients with condyloma acuminatum were examined. Complement fixing antibodies to wart virus were not demonstrable in any of these patients.

The applied technique and two-fold dilution of the serum samples, followed by reading as stated above, involves an analytical error of one dilution step, upwards or downwards. Consequently, weakly positive reactions may escape recognition and it was therefore found justified to include the dilution 1 : 4 among the titre values.

According to the available publications, the range of incidence of warts in a population is very wide, but generally it has been found to range between 3% and 6% (16). The incidence is highest among individuals in the age group 12 to 14 years. So far, it remains obscure whether resistance is acquired later in life or conditions of exposure change. Some occupational groups are particularly exposed (dermatologists, needlework mistresses) and subjects in these groups are more prone than others to be infected; not infrequently, they may be afflicted with multiple warts; even subjects in the age group 40 to 50 years may be susceptible. Owing to the small number of patients included in the individual age groups it has not been possible to analyse the incidence at which wart virus antibodies occur in subjects in the various age groups examined in the present study, but it is remarkable that even the oldest age groups were found to include a few positive cases. The percentage of CF-positive wart virus samples obtained in the group of donors (about 3%) is compatible with the incidence of warts in the population at large. Because of the nature of the present series of donors it has not been possible to embark upon a more detailed anamnestic analysis. The age at which patients included in the present series were still found to be susceptible to infection ranged higher by 4 or 5 years than ages hitherto recorded (16). The explanation is probably that surgical measures are practicable only in cases of older children and adult patients and thus, a few patients in whom antibody was demonstrable although they belonged in the older age groups, have been included; since the incidence of warts was highest among children, however, it proved impossible to examine a sufficient number of elderly subjects. Consequently, the series of patients and that of controls are hardly comparable.

Since available material is scarce, it has not been possible to compare qualitatively the coincident occurrence of antibody to wart virus with the presence of wart virus antigen demonstrable by fluorescent microscopy or biopsies obtained from one and the same patient (12, 16). So far, a quantitative comparison is also out of reach.

APPENDIX

A total of 249 blood samples from patients with warts were studied. Blood groups, determined according to the ABO-system, were distributed as follows:

Blood group	No. of samples	%	(Normal %)
O	105	43	(40)
A	102	41	(44)
B	32	13	(10–11)
AB	9	4	(4–5)

The finding of a higher number of group-B samples is not statistically significant if correlated with findings in the population at large.

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