COMPLEMENT FIXATION STUDIES WITH VERRUCA VULGARIS

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Janet W. Russell

1961

ABSTRACT

COMPLEMENT FIXATION STUDIES WITH VERRUCA VULGARIS

by Janet W. Russell

Serological experiments with verruca vulgaris were undertaken to substantiate findings that a wart agent was being passed in tissue culture and to determine to what extent antibodies to warts exist in the sera of patients. Complement fixation was the test indicated for these experiments.

Antisera to wart materials, both from infected tissue culture and wart tissue extracts, were prepared in rabbits by intravenous or subcutaneous injection. Antisera were also prepared against normal skin and normal AU cells as controls. Cross complement fixation tests using all antisera and all antigens were performed.

Findings indicated that an antigen quantitatively different from normal skin was present in both infected tissue culture materials and in wart tissue. This antigen could be assumed to be a wart agent. The cross reactions which occurred with normal skin indicated a close, probably inseparable relationship between the wart agent and its host cell.

Sera from eighteen persons, mainly patients whose warts were removed in the laboratory, were tested for antibodies to warts. Two of the patients were found to have antibodies against concentrated wart tissue antigen. Most patients did not have antibodies to warts, at least not in levels detectable by complement fixation tests with

concentrated antigens.

It was not ascertained in this study what part the presence of antibody plays in the regression of warts. The possibility that antibody is important in the regression of warts could not be discounted.

COMPLEMENT FIXATION STUDIES WITH VERRUCA VULGARIS

By

Janet W. Russell

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Microbiology and Public Health

1961

ACKNOWLEDGMENTS

The author wishes to express thanks to Dr. Walter N. Mack for his very patient guidance throughout this study.

Sincere thanks are also extended to the other workers in this laboratory, Joyce Remsberg, Barbara Riegle, James Frey, and Hajime Hayashi, for their assistance, helpful suggestions and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

An increasingly important area in cancer research is the study of virus-induced tumors. Numerous animal tumors have been shown to be caused by viruses, but human tumors have yet to be definitely established as virus-caused.

Among the tumors of man, verruca vulgaris, or common warts, has been considered to be of viral etiology since the end of the last century, but the specific causative agent has not been isolated and characterized up to this time. Experiments still in progress in this laboratory have shown that material from warts will produce cytopathogenic effect in tissue culture, specifically in AU cells, a human skin epithelial cell line. These results indicate that a specific wart agent is present. Cells from infected tissue culture can successfully passage the agent to new cells. The cells themselves are necessary to infect the tissue culture cells. Cytopathogenic effect has not been shown to be produced by cell-free nutrient fluid alone, as is the case with most viruses. The isolation of this agent is of importance both as further evidence of the viral etiology of tumors and as a means of providing tools for research into the causes of other tumors, both benign and malignant.

Complement fixation studies have been instigated to prove that the agent being passed in tissue culture is a specific wart agent. Complement fixation, while not an infallible technique, is the best method now available for this purpose. Human warts have not been transmitted to any experimental animal, and the hazards are too great

to attempt the inoculation of humans with the tissue culture material. Since human serum is part of the nutrient fluid used to propagate the tissue culture cells, there is danger of infecting humans with hepatitis virus or other viral contaminants present in a number of human sera. There is also no assurance that the so-called normal skin cell line has not acquired neoplastic properties. The inoculation of neoplastic cells into humans constitutes a risk.

Complement fixation, on the other hand, is a simple and specific technique, well-suited to viral antigens. The technique has also been widely used in tumor research. It must be noted, however, that complement fixation, or any other serological test used with tumors, even those known to be caused by viruses, presents a number of difficulties. The tumor agent may become an integral part of the cell and may not be transmitted without the cell. Such agents may incorporate antigenic components of the cell so that antibodies are formed against these normal cell components as well as against the antigens peculiar to the tumor agent. It is often impossible to obtain an antibody preparation specifically reactive against the tumor agent free of normal cell antibodies. Thus serological tests often show a quantitative, not qualitative, difference between infected material and normal tissue (Hauschka, 1952; Zibler, 1958).

In addition, other antigenic entities associated with normal cells, such as species-specific, organ-specific, and Forssman antigens may also complicate the picture. Tumors may also be accidentally infected with oncolytic viruses or other microbial agents which may lead to false results.

Serological work with tumors, although somewhat uncertain, has been increasingly successful in recent years. Results of complement fixation studies with other tumors warrant the assumption that complement fixation experiments with verruca vulgaris at this time can provide information valuable to the study of virus-caused tumors.

LITERATURE REVIEW

In spite of the difficulties involved in serological studies with tumors, a quantity of evidence has been amassed to suggest that tumor agents can be serologically identified. There have been no reports of verruca vulgaris having been studied previously with any serological technique, but the complement fixation reaction has been used by a number of investigators with a variety of human and animal tumors.

The early emphasis in complement fixation work with tumors was placed on cancer diagnosis. In 1908, Simon and Thomas tested the sera of patients with various malignant and non-malignant tumors against a human breast cancer antigen prepared by grinding tissue with phenolized saline solution. The results of their experiments showed complement fixation occurring in sixty-five per cent of the malignant cases and rarely in non-malignant cases. Their findings led them to believe that a positive complement fixation reaction with the "cancer antigen" was strong evidence of malignant disease.

Maderna (1935) performed complement fixation tests with sera from patients with venereal warts, condilomi acuminati, and found that complement was fixed in some cases.

Beginning in the 1930's Kidd and others did much work using complement fixation with the virus causing papillomas in rabbits, a condition similar to warts in man. The papilloma virus can be readily revealed by the complement fixation reaction since it is more nearly free of normal tissue components than most tumor viruses.

Kidd (1938a) found that the sera of rabbits bearing virus-induced papillomas fixed complement when mixed with extracts or filtrates of growths containing the virus. The papilloma antigens did not fix complement when mixed with the sera of normal rabbits, rabbits immune to other viruses or rabbits with papillomas induced by tarring. In these experiments, the complement binding antibody and the virus-neutralizing antibody seemed to exist in the same amount, both to the greatest degree in rabbits that had borne large growths over a long period of time. In some cases, sera that would neutralize small amounts of the virus would not fix complement.

In other experiments, Kidd (1938b) found that the complement fixing antigen of rabbit papillomas could be readily extracted from those growths yielding infectious virus but not from normal rabbit skin. The results of ultrafiltration and ultracentrifugation experiments showed the complement fixing antigen to have the same particle size as the infectious agent. Both could be destroyed by the same amount of heat or change in pH; however, it was possible to render the virus non-infectious with ultraviolet radiation or weak alkali without destroying the complement fixing antigen. From these experiments with rabbit papillomas, Kidd (1938c) concluded that the complement fixing and neutralizing antibodies were probably manifestations of a single antibody, the neutralization reaction having the lower threshold. In addition, it appeared that the infective virus and the complement fixing antigen were extremely closely related if not identical.

In 1940 Friedewald and Kidd carried out additional experiments

to show that the rabbit papilloma virus, itself, could elicit an antibody that would neutralize the virus and fix complement with virus preparations. There was no evidence of the "soluble antigen" separate from the infectious particle which is found associated with some viruses. The results were obtained from parallel neutralization and complement fixation tests.

In 1940, Bryan et al. performed complement fixation experiments with purified rabbit papilloma protein. They found that there was a close relationship between the infectivity and the complement binding power associated w ith the protein. Thus, they concluded that complement fixation tests may be used as biological assays of papilloma protein and as an indirect measurement of infectivity.

Hoyle (1940a) verified the work of Kidd. He found complement fixing antibody in the sera of sixteen of twenty-six domestic rabbits bearing papillomas. No such antibody was found in the sera of twenty normal rabbits. The complement fixation titers were low—ranging from 1:2 to 1:16. Tests performed at different times showed that the antibody appeared shortly after the appearance of the tumor and increased in amount as the tumor enlarged. By ultrafiltration and acetic acid precipitation experiments, Hoyle showed that the infective virus and the complement fixation antigen were the same.

Another rabbit tumor, the Brown-Pearce carcinoma, a highly malignant epithelioma arising in the testicles of rabbits, was studied by Kidd (1940a). He demonstrated a substance in saline extracts of the tumors capable of fixing complement when mixed with the sera of rabbits with the tumors. The substance was not

found in extracts of normal rabbit tissues, induced virus papillomas, induced uterine cancers of rabbits, or in rabbit tissues infected with certain viruses. Conversely, the sera of normal rabbits or of rabbits with various other conditions did not fix complement when mixed with extracts of the Brown-Pearce tumor. Kidd (1940b) also studied the nature of the serologically active substance of the Brown-Pearce carcinoma, the complement fixing antigen, and found it to be of uniform particle size as large as many of the viruses. It was sharply differentiated from the "soluble antigens" and resembled in some respects the papilloma virus.

Kabat and Furth (1940) found that the agent producing leukosis and sarcoma of fowls reacted with specific rabbit antisera in complement fixation and precipitation tests. Normal spleen also reacted with the anti-tumor sera in the two tests. The results of these experiments pointed out the problem of normal tissue components as integral parts of tumor agents.

Hoyle (1940b) described a complement fixing antigen derived from certain mouse tumors which appeared to be lipoid in nature. The antigen was an alcoholic extract of the tumors. Complement fixation occurred when the antigen was tested with the sera of mice bearing the tumors. No similar antigen could be extracted from normal mouse tissue, nor did normal mouse sera contain antibodies against the tumor antigen. Hoyle reported that Hirszfeld and Huhler had found that alcoholic extracts of human tumors sensitized with cholesterol gave complement fixation with a large proportion of sera from cases of malignant disease. Some non-specific reactions did

occur with sera from patients with syphilis or certain other diseases or from patients who were pregnant.

In 1941, Teixeira and Smadel found two "soluble antigens" in infected tissue of rabbits with infectious myxomatosis. The antigens retained their ability to fix complement after heating, but lost their specific precipitability.

Kidd and Friedewald (1942a) reported a "natural antibody" in the serum of normal rabbits which would fix complement when mixed with fresh saline extracts of normal rabbit tissues. They (1942b) ascribed the phenomenon to a naturally occurring serum principle which reacts specifically with a sedimentable constituent of normal tissue cells. The constituent with which the antibody reacted was present in a number of normal tissues, most abundantly in kidney and liver.

Further study of the Brown-Pearce rabbit carcinoma was carried out by MacKenzie and Kidd (1945). An antibody was found in the sera of rabbits implanted with or injected with Brown-Pearce carcinoma which would fix complement with a saline or aqueous extract of the tumor. The antibody was often of high titer in infected rabbits but was absent in the sera of normal rabbits or those with other types of tumors. The distinctive reactive substance in the tumors was found to differ notably from other substances extracted from various normal rabbit tissues.

The complement fixation reaction was also used by Malmgren et al. (1951) to study microsome fractions isolated from normal and neoplastic tissues of inbred mice. A quantitative difference was demonstrated

between normal liver and hepatomatous liver when reacted against antisera prepared against the hepatoma.

In experiments with mouse mammary tumor, Dmochowski and Passey (1952) found no qualitative difference between normal and agent-bearing tissue. Complement fixation tests revealed serological similarity between agent-free tissue and preparations of the agent.

In 1952, Rous, Kidd, and Smith performed complement fixation and neutralization experiments with an anaplastic epidermal rabbit carcinoma which had originated from a virus papilloma. During the first three and one-half years after its origination, the carcinoma had been passed twenty-two successive times. During this period rabbits bearing the carcinoma had been immune to the papilloma virus, and the sera of those rabbits were able to fix complement with papilloma virus. However, when tested after eight years of successive transfers, the carcinoma-bearing rabbits were no longer immune to papilloma virus, nor did their sera fix complement with virus preparations. There was no perceptible changein the tumor itself. The investigators concluded that the virus could not be the actuating cause of the carcinoma.

Eckert et al. (1955) found that the virus causing avian erythromyeloblastosis contained intrinsic constitutional elements antigenically indistinguishable from normal chicken tissue and Forssman antigen. Cross serological tests showed that sera against the normal tissue and Forssman antigen would effectively neutralize the virus and react in complement fixation tests with it. Along the same line, Beard, et al. (1955) concluded that the infective agent of avian erythromyeloblastosis consisted of a normal host antigen, a Forssman antigen and a third substance peculiar to the virus. The close

kinship of the virus to the host cell seemed obvious. The investigators postulated that similar relationships between tumor agents and their hosts may account for the difficulty encountered in isolating and serologically identifyimg tumor viruses.

Human tumors were the subject of investigation by Grahan and Grahan (1955). Complement fixation tests were performed with the sera of tumor patients against water-soluble, saline-insoluble fractions from their own tumors. Twelve of forty-eight patients showed complement fixing titers of from 1:16 to 1:128 in these tests. The majority of those who did not show antibodies had far-advanced tumors.

In 1959, Gardash'ian demonstrated complement fixing antibodies in the sera of twenty-three of thirty-nine rabbits with Brown-Pearce carcinoma. A protein fraction of the tumor was used as the antigen. In ninety per cent of the rabbits immunized with Brown-Pearce tumor tissue, the complement fixation reaction was positive.

Nartissov (1959) reported that complement fixation occurred when the sera of rabbits with papillomas were reacted with an antigen derived from a carcinoma which had originated from a virus papilloma. The results of his experiments led Nartissov to conclude that an antigenic complex existed in the carcinoma tissue consisting of the papilloma virus and the new malignant tissue.

Nartissov et al. (1959) also adsorbed papilloma virus onto red blood cells and demonstrated the phenomenon by the use of the complement fixation reaction. Rabbits were inoculated with their own red blood cells which had been adsorbed with papilloma virus.

The resulting antiserum fixed complement when tested with the virus-adsorbed cells. Complement fixation did not occur when red blood cells alone were reacted with the test sera.

The attempt to purify tumor antigens free of host tissue has continued to be a challenge to investigators. In 1959, Taylor et al. used fluorocarbon to purify complement fixing antigens from four chicken and human neoplastic tissues. He used Rous chicken sarcoma, a germ-free chicken fibrosarcoma, a human ovarian sarcoma, and human epithelial tissue culture cells to prepare the antigens. Crude antigens prepared from twenty per cent water suspensions of the tissues were treated with the fluorocarbon in a mixing blender then ultracentrifuged. The purified antigens were inoculated into rabbits. Antisera produced were specific to the respective tissues in complement fixation tests. Cross reactions with normal tissues did not occur. This method is reported to remove host lipid and protein leaving the tumor agent or complement fixing antigen free. Fluorocarbon purification was first described by Gessler et al. (1956) who used the method to separate a suspension of vaccinia virus from the chorio-allantoic membrane material of embryonating chicken eggs. The method was subsequently used by Manson et al. (1957) in purifying poliovirus after HeLa cell cultivation. Hummler and Hamparian (1957) also used fluorocarbon to remove anticomplementary substances and host antigens from poliovirus grown in HeLa and monkey kidney cells. Halonen et al. (1958) prepared enteric cytopathogenic human orphan (ECHO) complement fixing antigens from monkey kidney tissue culture cells by fluorocarbon purification.

The growing use of tissue culture antigens has enabled the complement fixation reaction to be employed with greater success in virus research. Anticomplementary factors are not encountered as often with tissue culture antigens as they are with virus-infected tissues themselves. Recently investigators have used tissue culture to propagate tumor agents (Eddy et al., 1958b; Beaudreau et al., 1958; Stewart et al., 1958). As a result, tumor agents in tissue culture have been used as complement fixing antigens.

The use of tissue culture antigens in the complement fixation reaction was demonstrated by Rowe et al. (1958) in studies with the Stewart- Eddy polyoma virus, the causative agent of mouse parotid gland tumors. These investigators found that the polyoma virus was serologically unrelated to a wide variety of known viruses. Tissue culture-grown polyoma virus was also used by Rowe et al. (1959a) in comparative complement fixation and hemagglutination inhibition tests. Eight mouse colonies were surveyed for the prevalence of antibody to polyoma virus. Positive hemagglutination inhibition titers ranged from zero to eighty-four per cent in adult mice in different colonies, and complement fixation results were comparable.

Neutralization tests have also been extensively used in tumor research. Neutralization studies have often been correlated with complement fixation tests. The ability of an antiserum to fix complement with a specific tumor antigen may or may not be associated with the ability of that serum to neutralize the tumor agent. The complement fixing antigen may be identical with the infective agent or may be a so-called "soluble antigen" distinct from the agent.

Thus antibodies elicited by the complement fixing antigen may neutralize the agent as in the case of the rabbit papillomas (Friedewald and Kidd, 1940) or may be unrelated to the infective ability of the agent, as in the case of the fowl sarcomas and leukoses (Kabat and Furth, 1941).

In early neutralization studies, Andrewes (1933) showed that sera from fowl bearing fibrosarcomata MH1 and CT10 would neutralize filtrates of several other fowl tumors. The fowl tumor viruses were found to be antigenically related but not identical.

Foulds (1937) produced antibodies in rabbits which neutralized filtrates of Rous sarcoma I, a chemically-induced fowl tumor. He concluded that a virus was present in an intracellular virus-cell protoplasm complex.

Cheever and Janeway (1941), investigating Brown-Pearce carcinoma, showed that rabbits with intradermally or subcutaneously implanted tumors which had regressed spontaneously, were immune to subsequent challenge implantation of tumor cells. However, it was found that implantation of homologous normal tissues would also confer some degree of immunity. The higher degree of immunity conferred by tumor cells implied that both a non-specific and a specific tumor factor were involved in eliciting the immune response.

Kabat and Furth (1941) produced neutralizing antibodies against fowl sarcoma and leukosis by injecting heavy tissue materials from the tumors into rabbits. Similar materials from normal spleen produced no neutralizing antibodies. In these experiments, the complement fixing antibody was found to be unrelated to the neut-

ralizing antibody. Complement fixing antibodies could be absorbed to a suspension of cells from normal chicken spleen. Antisera against materials from normal chicken spleen fixed complement in high dilutions but did not neutralize the agent. Sera of chickens immune to the viruses of leukosis and sarcomas contained neutralizing but not complement fixing antibodies.

Saphir et al. (1941) found that rabbits immunized to Brown-Pearce carcinoma by subcutaneous implantation developed tumors in the anterior chamber of the eye although all other tissues of the rabbit were resistant. The investigators concluded that there was a barrier between the aqueous fluid of the body and the blood carrying the circulating antibody which allowed the tumor to grow in the eye.

Gottschalk (1943) reported on a chemically induced chicken tumor which contained an antigen related to that of a fowl leukosis-sarcoma agent. High speed sediments of the tumor, when injected into rabbits, produced antibodies which would neutralize the leukosis-sarcoma agent after absorption with normal chicken spleen. Rabbit antisera to normal fowl tissue lacked the property to inactivate the fowl cancer agent.

Green et al. (1946) used neutralization tests to study the cancer "milk agent" (the Bittner virus) in mice. They found the agent stimulated the formation of antibodies in rabbits and rats. Such antisera neutralized mouse mammary carcinoma tissue. Normal rabbit and rat sera had a slight neutralizing effect on the carcinoma, but antisera against normal mouse tissue did not adversely affect

the agent. These findings pointed to exogenous origin of the virus. These results were disputed by later investigators who were not able to demonstrate a specific "milk agent" antigen free of host tissue.

Kidd (1946) investigated the antibody produced in rabbits implanted with Brown-Pearce carcinoma or with cell-free extracts of the tumor. From the results of neutralization tests, he concluded that the antibody reacted with a distinctive cell constituent which played a significant part in the proliferative activities of the Brown-Pearce tumor cell.

Duran-Reynals and King (1947) found that immune bodies developed naturally in the blood of aging chickens which would neutralize the virus of chicken tumors such as Rous sarcoma. These naturally occurring immune bodies appeared to be similar to the "natural antibodies" described by Kidd and Friedewald (1942 a, b).

Contrary to the work of Green et al. (1946), Gorer and Law (1949) could not clearly demonstrate specific neutralizing antibodies to the mammary tumor "milk agent."

Law and Malmgren (1951) also measured the neutralizing titer of mouse mammary tissue, both normal and malignant, which either contained or was free of the tumor agent. No consistent difference was found in the neutralizing titer of the antisera, implying the lack of a specific tumor antigen.

The problem of the incorporation of normal cell components as part of a tumor agent was encountered by Beard et al. (1957). Neutralization and precipitation tests were performed with concentrates of the virus of avian erythroblastosis. Chickens immunized with

these concentrates produced antibodies that effectively neutralized the virus and reacted with the virus in precipitation tests. However, the virus was neutralized by antiserum prepared in rabbits against normal chicken tissue. Antiserum against the virus of myeloblastosis was also effective in neutralizing the virus of erythroblastosis. Reciprocal neutralization tests showed the viruses of erythroblastosis and myeloblastosis to be antigenically similar. Some difference was evident in precipitation tests. These results indicated that the two agents were closely related but not identical.

In addition to complement fixation and neutralization tests, precipitation tests have also been used in the study of tumor agents. Mann and Welker, in 1940, reported producing an antiserum which would react in precipitation tests with the protein of human carcinoma but not with a large number of representative normal tissues.

Smadel et al. (1940) reported isolating two soluble antigens from skin and sera of rabbits acutely ill with myxomatosis. The two antigens could be separated by ammonium sulfate precipitation. Both reacted in precipitation tests with specific antibody.

Barrett (1941) used the precipitation reaction to test a particulate infectious fraction isolated from fowl sarcoma I with rabbit antiserum prepared against the fraction. Barrett was not able to show a difference between the sarcoma fraction and control tissue from an eight-day-old chicken embryo in these precipitation tests.

Imagawa, et al. (1948) found a quantitative difference, manifested in the precipitation reaction, between mouse mammary

tissue containing the cancer "milk agent" and tissue lacking the agent. Rabbit antisera against mammary cancer tissue reacted in high dilution with tissue containing the agent, whether normal or malignant, and in low dilution with tissue lacking the agent. Antisera prepared against normal mammary tissue abundant with milk agent reacted in the same way. Antisera against normal mammary tissue lacking the agent would precipitate the mammary agent in low dilutions. These results substantiated the neutralization findings of Gorer and Law (1949) and Law and Malmgren (1951) which indicated a close relationship between the "milk agent" and normal tissue.

Penn (1950) investigated the concept that some tumor antigens may be lipoid in nature. He found that lipoid fractions from human cancerous livers and tumor-bearing tissues would produce a flocculation when mixed with the sera of cancer patients. Similarly prepared livers of non-cancerous patients did not cause a flocculation. Penn hypothesized specific antibody formation in response to an endogenous carcinogen.

Some tumor viruses can be adsorbed onto red blood cells. Bittner (1947) found that the virus of the mammary cancer of mice, the "milk agent," could be adsorbed by the red cells of non-cancerous mice. Bittner (1945) had previously demonstrated that the mammary cancer virus was concentrated more in the blood cells of infected mice than in the serum.

Recent investigations have shown that the Stewart-Eddy polyoma virus would cause hemagglutination of erythrocytes. Eddy et al. (1958a) reported that polyoma virus would agglutinate red blood cells

and that immune serum would inhibit the hemagglutination. Hartley and Rowe (1959) reported that heating polyoma virus suspensions at 56 C for thirty minutes or treating the suspensions with a receptor destroying enzyme would allow hemagglutination to occur when it would not previously.

Rowe et al. (1959b) also used hemagglutination inhibition as a means of titrating and detecting the polyoma virus. Hemagglutination inhibition was found to be comparable in sensitivity, reproducibility, and time required to obtain results to the method employing the production of cytopathogenic effect in mouse embryo tissue culture.

Law et al. (1960), investigating the supposed relationship of polyoma to leukemia in mice, found that mice with hemagglutination inhibition titers to polyoma were not necessarily immune to leukemia. Any relationship between polyoma and leukemia in mice appeared to be an accidental one.

Neither tissues from human warts nor the agent causing human warts has been studied by serological tests. There has been no definite evidence whether or not antibodies against wart tissue exist. It is known clinically that children with warts can usually look forward to their regression at puberty (Blank and Rake, 1955). The question then arises whether the regression is the result of immunity to the agent causing warts or of some other yet unknown factor.

References were made earlier to the dangers of introducing viable tissue culture material into humans. Although an agent has been isolated in tissue culture from wart material, was it the agent causing warts? Here again the serological approach might contain the answer. This thesis presents the results of such an effort.

MATERIALS AND METHODS

PREPARATION OF ANTIGENS:

Tissue culture antigens: Three kinds of tissue culture antigens were used in these experiments: (1) wart-infected AU cells contained in nutrient fluid, (2) cell-free fluid from wart-infected AU cultures, and (3) normal AU cells contained in nutrient fluid (control). The AU cell line was originated by Wheeler et al. (1957) and has been serially passed in this laboratory for several years. Infected fluids and cells were prepared from AU cell cultures by first seeding with fragments of human wart tissues, then serially passing the infected cells to normal cells in culture. Infection was allowed to progress until most of the cells were detached from the glass surface. Remaining cells were detached with a rubber-tipped glass rod. When the fluid phase alone was used, the cells were sedimented by slow speed centrifugation. Uninoculated AU cell cultures were the normal AU cell controls. Monolayers were scraped off the glass surface with a rubber-tipped glass rod and the cells were suspended in the nutrient fluid. The nutrient fluid used to grow the cells was Hanks' basal salt solution (Hanks and Wallace, 1949) with 0.1 per cent yeast extract and 0.35 per cent glucose, containing 100 micrograms of streptomycin and 100 units of penicillin per milliliter. The pH indicator was phenol red, used in a concentration of 0.002 per cent. Growth medium was supplemented with twenty per cent inactivated human serum. Maintenance medium used with inoculated cultures was supplemented with two per cent inactivated calf serum. Tissue

culture antigens were stored at 4 C until used.

Tissue antigens: Extracts of human wart tissue and human normal skin were used as antigens. Human warts were removed by total enucleation from patients in the laboratory. Tissues were placed in Petri dishes and covered with approximately ten ml of Hanks' basal salt solution containing 100 micrograms of streptomycin and 100 units of penicillin per milliliter. The tissues were kept in this manner in the refrigerator at 4 C for at least twenty-four hours. This period was usually sufficient to free the tissues from bacteria.

Wart tissues were also removed from patients in the offices of physicians and immediately sealed in vials and frozen. Vials were sent to the laboratory in dry ice and stored at -20 C.

Fresh or frozen warts were mixed in a ten per cent suspension with saline solution or tissue culture nutrient medium. The mixtures were then ground with a pestle in a mortar for five minutes or placed in a mixing blender for four one-minute cycles. The liquid was then poured off into a conical centrifuge tube and centrifuged at 2000 rpm for ten minutes. The resulting supernatant fluid constituted the antigen. Merthiolate, in a concentration of 1:10,000, was used as a preservative when the antigens were prepared with saline solution. The tissue culture nutrient medium routinely contained antibiotics.

The human normal skin used was obtained from the Edward Sparrow Hospital. Foreskins from newborn infants were collected daily and stored at 4 C. until they were delivered to the laboratory. Ten

per cent saline suspensions of normal human skin were treated in the same manner as the wart tissue to prepare the normal skin antigen. The normal skin antigen was sealed in glass ampules, frozen, and stored at -20 C until used.

For use in some complement fixation tests, both the wart tissue antigen and the normal skin antigen, after the above treatment, were concentrated in the ultracentrifuge. The antigens were spun at 42,050 rpm (114,610 x g.) for one hour. Pellets were resuspended in one-sixth volume of the supernatant fluid. Concentrated antigens were stored at 4 C until they were used.

PREPARATION OF ANTISERA:

Rabbit antisera: The following antigens, prepared as described above, were used to prepare antisera in rabbits: (1) wart-infected AU cells in nutrient fluid, (2) cell-free nutrient fluid from wart-infected AU cultures, (3) extracts of wart tissue, and, as controls, (4) normal AU cells in nutrient fluid and (5) extracts of normal human skin. The antigens were inoculated into adult white rabbits three times weekly for three weeks. Rabbits receiving antigens containing cells were inoculated subcutaneously with 1.0 ml of the antigen at each injection. These rabbits received a total volume of 9.0 ml of antigen during the series of inoculations. Rabbits receiving cell-free fluids were inoculated intravenously by the marginal ear vein according to the following schedule: 0.5 ml, 0.75 ml, 1.0 ml, 1.0 ml, then 1.5 ml for each of the following

injections. These rabbits received a total volume of 10.75 ml of inoculum during the series. Rabbits were bled by heart puncture prior to inoculation and from ten days to two weeks after the final injection. At each bleeding, 20 ml of blood was taken and the serum was separated. Sera were inactivated at 56 C for thirty minutes, frozen and stored at -20 C until used. The rabbits were given booster injections, 1.0 ml of antigen subcutaneously, at weekly intervals for four weeks after the first post-inoculation bleeding and were bled subsequently as serum was needed.

Patients' sera: Approximately 10 to 20 ml of blood was taken from patients in the laboratory and the serum was separated. All sera were inactivated at 56 C for thirty minutes, then frozen and stored at -20 C until used. Sera were reinactivated for ten minutes at 56 C if a week or more had elapsed between the first inactivation and use.

OTHER REAGENTS

Saline solution: An 0.85 per cent solution of sodium chloride in distilled water was used in preparing all reagents and in the complement fixation tests. The saline solution was made up in liter amounts and sterilized by autoclaving.

Complement: The complement used in these experiments was either a commercial glycerolized preparation* or fresh pooled guinea pig serum. The commercial preparation was stored undiluted at 4 C until

*Bacto-complement from Difco Laboratories, Detroit Michigan

used. Commercial complement could be kept at this temperature for several months. The pooled guinea pig serum was obtained by bleeding fifteen to twenty guinea pigs by heart puncture. The pooled serum was dispensed in 1.0 ml amounts into small tubes with rubber stoppers, frozen and stored at -20 C until used. Complement was titrated before each test in the manner described by Smith et al. (1948). In the complement fixation tests, complement was used in a dilution determined by the titrations to contain two units per tube.

Hemolysin: The hemolysin used in the complement fixation tests was a commercial glycerolized preparation*. Hemolysin could be stored undiluted in the refrigerator for several months. It could also be stored for several weeks in a saline dilution in the refrigerator without appreciable loss of potency. Hemolysin was used in a dilution determined by previous titration to contain two units per tube (usually 1:250 to 1:1000). Diluted hemolysin was titrated by the method described by Smith et al. (1948) approximately every two weeks.

Sensitized cells: Sheep erythrocytes were obtained by bleeding young sheep and collecting the cells in equal volumes of modified Alsever's solution (Kent, et al., 1946). The cells were stored in the refrigerator at 4 C until used. Erythrocytes could be stored in this manner for a month to six weeks. Before each test a volume of the cells was centrifuged at 1500 rpm for ten minutes, washed three times with 0.85 per cent saline solution, packed at 1500 rpm for ten minutes, and finally made up to a two percent suspension with saline solution.

*Bacto-antisheep hemolysin from Difco Laboratories, Detroit, Michigan

Sensitized cells were prepared by mixing a volume of the two per cent suspension of erythrocytes with an equal volume of diluted hemolysin. Sensitized cells wereincubated at 37 C for ten minutes before use in complement fixation tests.

COMPLEMENT FIXATION TESTS

Cross complement fixation tests were performed with all antigens and rabbit antisera. Patients' sera were also tested with all antigens. In later tests, those antigens routinely used to test patients'sera were concentrated in the ultracentrifuge.

Complement fixation tests were performed according to the method described by Carpenter (1956) using constant antigen concentrations and two-fold dilutions of antiserum. In the earlier tests, a total volume of 1.0 ml per tube was used. The total volume was reduced to 0.5 ml per tube in later tests to conserve reagents. All reagents were kept cold and tests were usually conducted in an ice bath. Antigens were used undiluted or diluted 1:2. Occasionally antigens were anticomplementary. Such antigens were titrated (Smith et al., 1948) to find the concentration at which they could be used in the test without anticomplementary activity. Sera were sometimes anti-complementary in high concentrations (undiluted, 1:2, 1:4). Serum controls were routinely run for those dilutions and for all dilutions with particularly troublesome sera. Two units of complement per tube were used, determined by complement titration before each test. Commercial complement was usually diluted 1:10 to 1:25. Frozen pooled guinea pig serum could usually be diluted 1:30 to 1:40.

Diluted complement was not used after standing more than a few hours.

Two periods of incubation were used in the complement fixation tests. The antigen-antiserum-complement mixtures were first incubated overnight (16 to 18 hours) at 4 C so that fixation could occur. The sensitized cells were then added and the tests were reincubated at 37 C for thirty minutes. In early tests, a primary incubation period of one hour at 37 C was employed instead of 16 to 18 hours at 4 C. The refrigeration method was found to yield more sensitive results, however, as reported by the Committee on Diagnostic Procedures for Virus and Rickettsial Diseases (1956).

Results were read immediately after the secondary incubation and finally after overnight refrigeration. Tubes showing no hemolysis were read as four plus. Varying degrees of hemolysis were read as three plus, two plus, one plus, or zero in the case of complete hemolysis. The endpoint was considered to be complete hemolysis.

ABSORPTION PROCEDURES

Rabbit antiserum against infected AU cells was absorbed with normal skin and normal AU cells in an attempt to remove antibodies against normal cell components. Antiserum against cell-free fluid from infected AU cultures was also absorbed with normal skin. For use in this procedure, normal skin was ground in a mixing blendor with saline solution for four one-minute cycles. The normal skin particles were then sedimented by slow-speed centrifugation. The supernatant fluid was removed. The sediment was mixed with an equal volume of inactivated antiserum, incubated one hour in a 37 C water-

bath, then overnight in the refrigerator at 4 C. The mixture was then centrifuged at 2000 rpm and the supernatant serum frozen.

The normal AU cells for use in absorption procedures were prepared by detaching the cells of a fully-sheeted monolayer from the glass surface and packing them by slow speed centrifugation. AU cells were then resuspended in saline solution in a concentration of approximately 900,000 cells per milliliter determined by counting crystal violet stained cells in a hemocytometer or by the turbidity of the suspension. The absorption procedure used was the same as that used for normal skin.

Absorbed sera were reabsorbed with normal skin or normal AU cells, prepared as above. Reabsorbed sera were frozen until used for complement fixation tests.

FLUOROCARBON TREATMENTS

Taylor et al. (1959) reported a method for purifying complement fixing antigens by the use of a fluorocarbon, Genetron 113*. This method was reported to remove normal tissue proteins and lipids from various human and chicken neoplastic tissues leaving a specific complement fixing antigen. Fluorocarbon purification has also been used by several other investigators to purify viruses (Gessler et al., 1956; Manson et al., 1957; Hummler and Hemperian, 1957). A modification of Taylor's method was tried in an attempt to remove normal tissue substances from wart tissue and wart-infected AU cells.

*Genetron 113 (trifluorotrichloroethane) obtained from Allied Chemical and Dye Corporation

Pieces of wart tissue, a ten per cent suspension in tissue culture nutrient medium, were ground in a morter. To two parts of the ground wart suspension, one part of Genetron 113 was added. The whole mixture was then ground for five minutes. The morter was kept cold in an ice bath during the grinding. The resulting liquid was poured off into a conical centrifuge tube and spun at 2000 rpm for ten minutes. Three layers were evident after centrifugation: a bottom layer of clear fluorocarbon, a thin middle layer of white solid material and a clear aqueous top layer. The aqueous layer was removed and constituted the treated antigen.

Wart-infected AU cells in nutrient medium were mixed with Genetron 113 in a prescription bottle, two parts of the cell suspension to one part of the fluorocarbon, and shaken vigorously for several minutes. The mixture was then transferred to a cold morter and ground with a pestle for five minutes. This ground mixture was returned to a prescription bottle and was shaken for five more minutes before being placed in a conical centrifuge tube. Centrifugation at 2000 rpm for ten minutes revealed the same three layers that were evident with the wart tissue. The top aqueous layer, constituting the treated antigen, was removed.

Antisera against the two treated antigens were prepared by inoculating rabbits intravenously by the same procedure used to prepare the other rabbit antisera.

RESULTS

Results of complement fixation tests with rabbit antisera:

Antisera were prepared in rabbits against the wart materials and control antigens described above. The antigens used, total volumes given, and routes of inoculation are summarized in table I. Those rabbits receiving the wart-infected AU cells and normal AU cells were given a total volume of 9.0 ml during the series of injections. These antigens, which contained whole cells, were given subcutaneously to avoid the formation of emboliams which might have occurred had they been given intravenously. Those rabbits which were given cell-free fluid from infected AU cells, extract of wart tissue, and extract of normal skin received a total volume of 10.75 ml of antigen intravenously. Complement fixation tests with sera obtained from pre-inoculation bleedings revealed that none of the rabbits used in these experiments had antibody titers to the test antigens prior to inoculation.

Table II presents the results of cross complement fixation tests in which the rabbit antisera were tested against all the prepared antigens. After the series of injections, all inoculated rabbits had developed antibodies against their homologous antigens. (The diagonal line connects the results with the homologous antigens.) These titers were low, ranging from 1:16 to 1:256. As expected, the cross complement fixation results also showed that the rabbits had developed, to a lesser degree, titers against all the other antigens. It can be seen, however, that rabbits inoculated with wart-infected

Table I. Rabbit inoculations

Rabbit	Antigen used	Total volume of inoculum	Route of inoculation
1	Wart-infected AU cells	9.0 ml	subcutaneous
2	Cell-free fluid from wart-infected AU cells	10.75 ml	intravenous
3	Extract of wart tissue	10.75 ml	intravenous
4	Normal AU cells	9.0 ml	subcutaneous
5	Extract of normal skin	10.75 ml	intravenous

antigens developed somewhat higher titers against the infected antigens than against the control antigens.

The first antiserum listed in table II, that produced against infected AU cells, had a titer of 1:64 when tested against the homologous antigen, infected cells. When this antiserum was tested against cell-free fluid from infected cultures, the titer was 1:32. The same titer, 1:32, resulted when the antiserum was tested against extract of wart tissue. In contrast, the titers of this antiserum against the control antigens were significantly lower: 1:4 against normal AU cells and 1:8 against normal skin.

The antiserum prepared against cell-free fluid from infected AU cultures had a titer of 1:128 against the homologous antigen. A titer of 1:64 resulted when this antiserum was tested against infected cells themselves. Against extract of wart tissue, the titer was 1:32. Again, in the case of this antiserum as with the previous one, titers were somewhat lower against the control antigens: 1:4 against normal AU cells and 1:8 against normal skin.

When the antiserum produced against extract of wart tissue was tested against the homologous antigen, the resulting titer was 1:64. Titers of this antiserum against the infected tissue culture antigens, however, were lower: 1:16 against infected cells and 1:16 against cell-free fluid from infected cultures. Titers against the control antigens were 1:8 against normal AU cells and 1:16 against normal skin.

The antisera prepared against the two control antigens, normal AU cells and normal skin, were also tested in complement fixation reactions against the five prepared antigens. The titer of the

Table II. Results of complement fixation tests with rabbit antisera

Rabbit antisera	Antigens				
	Infected AU cells	Cell-free fluid from infected AU cells	Extract of wart tissue	Normal AU cells	Normal skin
Infected AU cells	1:64	1:32	1:32	1:4	1:8
Cell-free fluid from infected AU cells	1:64	1:128	1:32	1:4	1:8
Extract of wart tissue	1:16	1:16	1:64	1:8	1:16
Normal AU cells	1:16	1:16	1:16	1:16	1:8
Normal skin	1:128	1:64	1:128	1:128	1:256

antiserum against normal AU cells was 1:16 when that antiserum was tested against the homologous antigen, normal AU cells. The same titer, 1:16, also resulted when that antiserum was tested against infected cells, cell-free fluid from infected cells and extract of wart tissue. Against normal skin, the titer was slightly lower, 1:8.

The antiserum prepared against normal skin showed generally higher titers against the various antigens than did any of the other antisera. The titer was 1:256 against the homologous antigen, normal skin. Against infected cells the titer of this antiserum was 1:128. Against cell-free fluid from infected cultures a titer of 1:64 resulted. The titer of this antiserum when tested against both extract of wart tissue and normal AU cells was 1:128.

Absorption results:

Antisera prepared in rabbits against infected AU cells and cell-free fluid from infected cultures were absorbed with normal skin in an effort to remove antibodies against normal tissues. The antiserum against infected cells was also absorbed with normal AU cells. Absorption procedures did not succeed in eliminating the antibodies against normal tissues completely. Complement fixation titers of these antisera were reduced one or two dilutions, both against the infected antigens and the control antigens. These results are summarized in Table III.

The antiserum against infected cells, when tested prior to absorption with normal skin had a titer of 1:32 against ultra-centrifuge concentrated wart tissue extract and 1:16 against

concentrated normal skin. After absorption twice with normal skin, the complement fixation titer against both the concentrated wart antigen and concentrated normal skin was 1:8.

When the antiserum against cell-free fluid from infected cultures was tested prior to absorption with normal skin, titers were 1:16 against concentrated wart tissue and 1:8 against concentrated normal skin. After the antiserum had been absorbed twice with normal skin, titers of 1:8 against concentrated wart antigen and 1:4 against concentrated normal skin were demonstrated.

The antiserum against infected cells was also absorbed with normal AU cells. Pre-absorption titers of this antiserum were 1:32 against infected AU cells and 1:8 against normal AU cells. After absorption, those titers had been reduced to 1:8 against the infected cells and 1:4 against the normal cells.

Results of complement fixation tests with patients' sera:

Since it is known clinically that warts are far less common after the second decade of life (Blank and Rake, 1955), it was postulated that adult gamma globulin might contain antibodies against warts. Commercial gamma globulin was tested in complement fixation reactions with the five antigens which were used to prepare antisera in rabbits. Gamma globulin proved to be anticomplementary in dilutions up to 1:128. Higher dilutions showed no complement fixation with the test antigens. The anticomplementary activity of gamma globulin has been reported by other investigators (Marcus, 1960).

Eighteen human sera were then tested for antibodies against wart materials. Twelve of the eighteen were from patients whose warts were removed in the laboratory. Of the remaining six, one serum was from an eighteen-month-old child (GH) without warts. Another was from a young woman in her twenties (JR) who had had warts as a child but had been free of them for a number of years. Another serum was from a young man in his twenties (JF) who did not ever remember having warts. The other three were unidentified adult sera which had been obtained from the Red Cross for use in tissue culture nutrient medium.

In early tests, the human sera were reacted with wart-infected AU cells, cell-free fluid from infected cultures, extract of wart tissue, normal AU cells, and extract of normal skin. None of the sera fixed complement with any of these antigens. It was then decided to concentrate the wart-infected AU cells and the extract of wart tissue in the ultracentrifuge. Normal AU cells and extract of normal skin were also concentrated as controls.

Two of the wart patients, DA and JP, who were bled after having warts removed in this laboratory, were found to have antibody titers against ultracentrifuge-concentrated extract of wart tissue. Both sera were consistently positive when tested with a number of batches of antigen prepared from either fresh or frozen warts. These sera were routinely used as positive controls in testing the sera of the other patients.

Serum from DA showed an antibody titer ranging from 1:8 to 1:32 with different batches of antigen. The titer of the serum from JP ranged from 1:8 to 1:16. Neither DA nor JP serum had complement

fixing antibodies against normal skin which had been concentrated in the ultracentrifuge as a control.

Sera from DA and JP were also tested against both infected AU cells and normal AU cells which had been concentrated in the ultracentrifuge. Neither serum could be shown to have complement fixing antibodies against either the infected or the control antigen.

These two patients were found to have had no recurrence of warts when checked some months later.

Results of fluorocarbon treatments:

Representative complement fixation tests were performed to ascertain whether fluorocarbon treatments had removed normal tissue substances from infected tissue culture and wart tissue. The results of these tests indicated that normal tissue substances were not removed by the treatments as outlined.

Antiserum prepared in rabbits against treated wart tissue had a titer of 1:64 against wart tissue and 1:16 against normal skin.

Antiserum prepared against fluorocarbon-treated infected AU cells had a titer of 1:16 when tested against normal AU cells.

Infected AU cells treated with fluorocarbon were used as the antigen in complement fixation tests with antiserum prepared against untreated infected AU cells. The resulting titer was 1:16. The titer obtained when this treated tissue culture antigen was tested with antiserum against normal AU cells was 1:8.

DISCUSSION

The results of the experiments with rabbit antisera have demonstrated a pattern similar to that shown by other serological investigations of tumors. The problems of imperfect specificity and overlapping reactions with normal tissue have constantly recurred in tumor studies. It has been possible in the past to produce antibodies against human tumor tissue in experimental animals, (Landsteiner, 1945) but there has continually been the problem of overlapping with normal tissue antigens.

These experiments have shown an antigenic component in both wart-infected tissue culture and in wart tissue extracts that did not occur in normal tissue. There were also common antigenic components shared by the infected and normal tissue as evidenced by the cross reactions which occurred with both normal AU cells and normal skin. Absorption procedures did not rid antisera against infected materials of normal cell antibodies. Fluorocarbon treatments were also unsuccessful in ridding antigen preparations of normal tissue antigens.

These findings and similar findings of other investigators in the tumor field were to be expected. Assuming that the extra antigenic components represented a causative agent of warts, such an agent would exist in close relationship with the host cell. Tumor agents may become integral parts of the infected cell and incorporate cellular components which may well be antigenic. These agents may not even be transmitted without passage of the host cell. That this was the case with the wart agent was indicated by the results of

other experiments which have been carried out in this laboratory with warts in tissue culture. An agent isolated from wart pieces which would produce cytopathogenic effect in tissue culture could be passaged to uninfected cell cultures if the infected cells themselves were passed. No cytopathogenic effect has been demonstrated when cell-free fluid from infected cultures was used as the inoculum. These results indicated a close relationship between the wart agent and the host cell. It was therefore highly unlikely that an antiserum specifically against the wart agent, free of normal cell antibodies, could have been produced.

A primary object in this investigation was to support the tissue culture findings. It was apparent from complement fixation tests with rabbit antisera that some antigenic component existed in infected tissue culture which was not present in normal tissue. Results with antisera produced against normal tissues supported this assumption. Titers were essentially the same when those antisera were tested against all antigens. These findings indicated that the antiserum was reacting with the normal cell elements of both the infected and control antigens. This provided further evidence that the infected materials contained an antigen not present in normal skin.

That it was the actual wart agent that was being passed in tissue culture was also supported by the results. Titers of antisera prepared against infected tissue culture materials were also higher against wart tissue extracts than against normal tissues.

It has been found that the whole infected cell is necessary to pass the agent in tissue culture. Still, cell-free fluid from

infected cultures apparently contained the antigen associated with the wart agent. The agent which may have been freed from destroyed cells or even denatured must still have been able to elicit the formation of complement fixation antibodies, even though it may take the intact cell containing the agent to infect. It has been found in work with other tumor agents that destruction of infectivity does not necessarily destroy the ability of an agent to elicit complement fixing antibodies (Kidd, 1938b).

Complement fixation titers with all the rabbit antisera were low. These titers compared favorably, however, with results obtained by other investigators in work with tumors and tumor agents (Kidd, 1938a; Rowe et al., 1958; Taylor, 1959; Graham and Graham, 1955). Some rabbits developed a higher range of titers than others. This might have been due to individual differences in the rabbits or to the route of inoculation. Those rabbits inoculated intravenously, in general, developed higher titers than those inoculated subcutaneously. The intravenous route was more conducive to the production of higher antibody levels. In addition, those rabbits inoculated intravenously actually received more antigen than those inoculated subcutaneously.

Complement fixation tests with patients' sera have shown that most wart patients did not have antibodies against the wart agent in detectable levels. It was not possible to show complement fixation with patients' sera and any of the routinely used antigens. When wart tissue extract that had been concentrated in the ultracentrifuge was used as the antigen, two of the eighteen persons tested proved to have complement fixation titers against that antigen.

The question of why most wart patients did not have detectable complement fixing antibodies against warts could be answered in part by the close relationship between the wart agent and the host cell, which results with tissue culture and antisera produced in rabbits have indicated. It was not likely that antibodies would be produced against the agent bearing cell--a part of the patient's own tissue. The agent itself might have been so closely protected by the cell that only small amounts were available to stimulate the production of antibodies. The agent might have existed only in small amounts in the cell. The level of antibody production in most patients might have been so low that it was not detectable in complement fixation tests even with concentrated wart antigen. In the case of some other patients, antigen might have been released in large enough amounts to produce a detectable antibody level.

The question was also raised as to what part wart antibodies play in the regression of warts. The patients who were found to have antibodies had had no recurrence of their warts some months after removal. However, this was not time enough on which to base any definite conclusions. The sera of JR whose childhood warts had spontaneously regressed was negative when tested against the concentrated wart antigen. Still, it is not possible to discount the possibility of an immunological mechanism of regression. The patient may have had an antibody titer against warts which had diminished during the years since the regression of the warts. An area for possible study would be patients whose warts were in the process of spontaneous regression.

It has been discussed that the agent might have been so closely related to the cell that it might not have been released in large enough amounts to elicit the formation of antibodies. It is also conceivable that antibody produced against the wart agent may not penetrate the cell because of the semi-permeability of the cell membrane. In that case, regression of warts may be due to some entirely different mechanism than the usual antigen-antibody reaction.

This study has provided serological evidence that a wart agent has been passed in tissue culture. Findings have also supported the assumption that the wart agent is closely related to the host cell and may contain antigenic components of the cell. A small percentage of wart patients have been shown to have complement fixing antibodies to warts. It has not, however, been determined what part the presence of antibody plays in the regression of warts.

SUMMARY

1. Antisera were produced in rabbits against wart-infected tissue culture materials and wart tissue extracts.

2. Complement fixation tests indicated that a wart antigen exists. A quantitative difference was found between the titers against infected materials and those against normal skin.

3. Results of cross complement fixation tests with rabbit antisera indicated that the same antigen exists in wart tissue extracts and in infected tissue culture. From this data it can be assumed that the actual warts agent has been passed in AV cell cultures.

4. Cross reactions with normal skin and normal AV cells could not be eliminated from infected materials by fluorocarbon treatments or from antisera against infected materials by absorption. It can be assumed that the wart agent contains components of the host cell.

5. Most of the wart patients tested had no complement fixing antibodies against wart materials. A small number of patients were found to have antibodies against warts in low level.

6. It was not determined what part, if any, that the presence or absence of antibodies plays in the regression of warts.

BIBLIOGRAPHY

ANDREWES, C. H. 1933 Further serological studies on fowl-tumor viruses. J. Pathol. Bacteriol., 37, 27-44.

BARRETT, M. Y. 1941 The antigenic nature of purified chicken tumor agent. Preliminary report. Cancer Research, 1, 543-544.

BEARD, D. W., G. S. BEAUDREAU, R. A. BONAR, D. G. SHARP, AND J. W. BEARD 1957 Virus of avian erythroblastosis. III. Antigenic constitution and relation to the agent of avian myeloblastosis. J. Natl. Cancer Inst., 18, 231-259.

BEARD, J. W., D. G. SHARP, AND E. A. ECKERT 1955 Tumor viruses. Advances in Virus Research, III, 149-193.

BEAUDREAU, G. S., C. BECKER, D. G. SHARP, J. C. PAINTER, AND J. W. BEARD 1958 Virus of avian myeloblastosis. XI. Release of the virus by myeloblasts in tissue culture. J. Natl. Cancer Inst., 20, 351-377.

BITTNER, J. J. 1945 Characteristics of the mammary tumor milk agent in serial dilution and blood studies. Proc. Soc. Exptl. Biol. Med., 59, 43-44.

BITTNER, J. J. 1947 The mammary tumor milk agent. Ann. New York Acad. Sci., 49, 69-73.

BLANK, H. AND G. RAKE 1955 Viral and rickettsial diseases of the skin, eye, and mucous membranes of man, pp. 156-181. Little, Brown, and Company, Boston

BRYAN, W. R., D. W. BEARD, AND J. W. BEARD 1940 The complement fixing capacity of the rabbit papilloma virus protein. J. Natl. Cancer Inst., 1, 197-203.

CARPENTER, P. L. 1956 Immunology and serology, p. 320. W. B. Saunders Co., Philadelphia.

CHEEVER, F. S. AND C. A. JANEWAY 1941 Immunity induced against the Brown-Pearce carcinoma. Cancer Research, 1, 23-27.

COMMITTEE ON DIAGNOSTIC PROCEDURES FOR VIRUS AND RICKETTSIAL DISEASES, AMERICAN PUBLIC HEALTH ASSOCIATION 1956 Diagnostic procedures for virus and rickettsial diseases. American Public Health Association, New York.

DMOCHOWSKI, L. AND R. C. PASSEY 1952 Attempts at tumor virus isolation. Ann. New York Acad. Sci., 54, 1035-1066.

DURAN-REYNALS, F. AND J. W. KING 1947 Reciprocal effects of natural immune bodies from chickens and ducks on variants of a sarcoma virus. Cancer Research, 7, 21-25.

EGERT, E. A., D. G. SHARP, S. W. BEARD, I. GREEN, AND J. W. BEARD 1955 Virus of avian erythromyeloblastic leukosis. IX. Antigenic constitution and immunologic characterization. J. Natl. Cancer Inst., 16, 593-634.

EDDY, B. E., W. P. ROWE, J. W. HARTLEY, S. E. STEWART, AND R. J. HUEBNER 1958a Hemagglutination with the Stewart-Eddy polyoma virus. Virology, 6, 290-291.

EDDY, B. E., S. E. STEWART, R. YOUNG, G. B. MYDER 1958b Neoplasms in hamsters induced by mouse tumor agent passed in tissue culture. J. Natl. Cancer Inst., 20, 747-762.

FOULDS, L. 1937 Observations on non-filterable fowl tumors. The production of neutralizing sera against filtrates of Rous Sarcoma I by non-infective extracts of a sarcoma induced by 1,2,5,6-dibenzanthracene. Am. J. Cancer, 31, 404-413.

FRIEDEWALD, W. F. AND J. G. KIDD 1940 Union in vitro of the papilloma virus and its antibody. J. Exptl. Med., 72, 531-558.

GARDASHIAN, A. M. 1959 Demonstration of antibodies in rabbits with Brown-Pearce tumors. In Pathogenesis and immunology of tumors, pp. 182-195. Edited by G. V Vygodchikov. Pergammon Press, New York.

GESSLER, A. E., C. E. REMDER, M. C. PARTINGTON 1956 A new and rapid method for isolating viruses by selective fluorocarbon deproteinization. Trans. New York Acad. Sci., 18, 701-703.

GOFFER, P. A. AND LAW, L. W. 1949 An attempt to demonstrate neutralizing antibodies to the mammary tumor "milk agent" in mice. Brit. J. Cancer, 3, 90-93.

GOTTSCHALK, G. R. 1943 Observations on a chemically induced chicken tumor containing an antigen related to that of a leukosis sarcoma agent. Cancer Research, 3, 649-667.

GRAHAM, J. G. AND R. M. GRAHAM 1955 Antibodies elicited by cancer in patients. Cancer, 8, 409-416.

GREEN, R. G., M. M. MOOSEY, AND J. J. BITTNER 1946 Antigenic character of the cancer milk agent in mice. Proc. Soc. Exptl. Biol. Med., 61, 115-117.

HALONEN, P., R. J. HUEBNER, AND H. C. TURNER 1958 Preparation of ECHO Complement-fixing antigens in monkey kidney tissue culture and their purification by fluorocarbon. Proc. Soc. Exptl. Biol. Med., 97, 530-535.

HAITS, J. F. AND R. E. WALLACE 1949 Relation of oxygen and temperature in the preservation of tissues by refrigeration. Proc. Soc. Exptl. Biol. Med., 71, 196-200.

HARTLEY, J. W. AND W. P. ROWE 1959 "Unmasking" of mouse polyoma virus hemagglutinin by heat and RDE. Virology, 7, 240-250.

HAUSCHKA, T. S. 1952 Immunologic aspects of cancer: a review. Cancer Research, 12, 615-633.

HOYLE, I. 1940a Complement fixation with the Shope rabbit papilloma virus. J. Pathol. Bacteriol., 50, 169-170.

HOYLE, I. 1940b A lipoidal antigen produced by certain malignant tumors of the mouse. Am. J. Cancer, 39, 224-233.

HUEBNER, K. AND V. V. HAMPARIAN 1957 Removal of anticomplementary activity and host antigens from viral preparations by fluorocarbon. Science, 125, 547-548.

IMAGAWA, D. T., R. G. GREEN, AND H. O. HALVORSON 1948 A precipitin test for antigens present in mouse tissues containing the milk agent. Proc. Soc. Exptl. Biol. Med., 68, 162-166.

KABAT, E. A. AND J. FURTH 1940 Chemical and immunological studies on the agent producing leukosis and sarcoma of fowls. J. Exptl. Med., 71, 55-70.

KABAT, E. A. AND J. FURTH 1941 Neutralization of the agent causing leukosis and sarcoma of fowls by rabbit antisera. J. Exptl. Med., 74, 257-261.

KENT, J. R., S. C. BUKANTZ, AND C. R. REIN 1946 Studies in complement fixation. I. Spectrophotometric titration of complement; construction of graphs for direct determination of the fifty per cent hemolytic unit. J. Immunol. , 53, 37-50.

KIDD, J. G. 1938a Immunological reactions with a virus causing papillomas in rabbits. I. Demonstration of a complement fixation reaction, relation of virus-neutralizing and complement binding antibodies. J. Exptl. Med., 68, 703-724.

KIDD, J. G. 1938b Immunological reactions with a virus causing papillomas in rabbits. II. Properties of the complement-binding antigen present in extracts of the growths; its relation to the virus. J. Exptl. Med., 68, 737-759.

KIDD, J. G. 1938c Immunological reactions with a virus causing papillomas in rabbits. III. Antigenicity and pathogenicity of extracts of the growths of wild and domestic species, general discussion. J. Exptl. Med., 68, 737-759.

KIDD, J. G. 1940a A distinctive substance associated with the Brown-Pearce rabbit carcinoma. J. Exptl. Med., 71, 335-350.

KIDD, J. G. 1940b A distinctive substance associated with the Brown-Pearce rabbit carcinoma. II. Properties of the substance; discussion. J. Exptl. Med., 71, 351-371.

KIDD, J. G. 1946 Suppression of growth of Brown-Pearce tumor cells by a specific antibody. J. Exptl. Med., 83, 227-250.

KIDD, J. G. AND W. F. FRIEDEWALD 1942a A natural antibody that reacts in vitro with a sedimentable constituent of normal tissue cells. I. Demonstration of the phenomenon. J. Exptl. Med., 76, 557-578.

KIDD, J. G. AND W. F. FRIEDEWALD 1942b A natural antibody that reacts in vitro with a sedimentable constituent of normal tissue cells. II. Specificity of the phenomenon; general discussion. J. Exptl. Med., 76, 579-588.

LANDSTEINER, K. 1945 The specificity of serological reactions, 2nd rev. ed. Harvard University Press, Cambridge.

LAW, L. W. AND R. A. MALMGREN 1951 Studies on the "cytotoxic" property of antiserum of the mammary tumor agent. J. Natl. Cancer Inst., 11, 1259-1268.

LAW, L. W., W. P. ROWE, AND J. W. HARTLEY 1960 Studies of mouse polyoma virus infection. V. Relation of virus infection to lymphocytic neoplasms of the mouse. J. Exptl. Med., 111, 517-523.

MACKENZIE, I. AND J. G. KIDD 1945 Incidence and specificity of the antibody for a distinctive constituent of the Brown-Pearce tumor. J. Exptl. Med., 82, 41-63.

MADERNA, C. 1935 La diviazione del complemento in infermi affetti da condilomi acuminati. Riforma Med., 51, 93-94.

MALMGREN, R. A., R. E. BENNISON, G. R. ANDERSON, AND C. C. RISLEY 1951 Serologic study of the microsome fraction of normal and neoplastic mouse tissues. J. Natl. Cancer Inst., 11, 1277-1286.

MANN, L. D. AND W. H. WELKER 1940 A specific precipitin antiserum for carcinoma protein. Am. J. Cancer, 39, 360-364.

MANSON, L. A., E. L. ROTHSTEIN, AND G. W. RAKE 1957 Purification of poliovirus with fluorocarbon. Science, 125, 546-547.

MARCUS, D. M. 1960 A study of the mechanism of the anticomplementary activity of gamma-globulin. J. Immunol., 84, 273-284.

NARTISSOV, N. V. 1959 The complement fixation reaction in relation to rabbit papilloma. In Pathogenesis and immunology of tumors, pp. 220-225. Edited by G. V. Vygodchikov. Pergamon Press, New York.

PENN, H. S. 1950 Tumor lipoids as antigens (preliminary report). Cancer Research, 10, 235.

ROUS, P., J. G. KIDD, AND W. E. SMITH 1952 Experiments on the cause of the rabbit carcinomas derived from virus-induced papillomas. II. Loss by the VX2 carcinoma of the power to immunize hosts against papilloma virus. J. Exptl. Med., 96, 159-174.

ROWE, W. P., J. W. HARTLEY, I. BRODSKY, AND R. J. HEUBNER 1958 Complement fixation with a mouse tumor virus (Stewart-Eddy polyoma). Science, 128, 1339-1340.

ROWE, W. P., J. W. HARTLEY, L. W. LAW, AND R. J. HEUBNER 1959a Studies of mouse polyoma virus infection. III. Distribution of antibodies in laboratory mouse colonies. J. Exptl. Med., 109, 449-462.

ROWE, W. P., J. W. HARTLEY, J. E. ESTES, AND R. J. HEUBNER 1959b Studies of mouse polyoma virus infection. I. Procedures for quantitation and detection of virus. J. Exptl. Med., 109, 379-391.

SAPHIR, O., M. APPEL, AND A. A. STRAUSS 1941 Growth of Brown-Pearce carcinoma in the anterior chamber of the eyes of tumor immune rabbits. Cancer Research, 1, 543-547.

SIMON, C. E. AND W. S. THOMAS 1908 On complement fixation in malignant disease. J. Exptl. Med., 10, 673-689.

SWADEL, J. E., S. M. WARD, AND T. M. RIVERS 1940 Infectious myxomatosis of rabbits. II. Demonstration of a second soluble antigen associated with the disease. J. Exptl. Med., 72, 129-138.

SMITH, D. T., D. S. MARTIN, N. F. CONANT, J. W. BEARD, G. TAYLOR, H. I. KOHN, AND M. A. POSTON 1948 Zinsser's textbook of bacteriology, 9th ed., pp. 941-942. Appleton-Century-Crofts, Inc., New York.

STEWART, S. E., G. E. EDDY, AND N. BORGESE 1958 Neoplasms in mice inoculated with a tumor agent carried in tissue culture. J. Natl. Cancer Inst., 20, 1223-1244.

TAYLOR, A. R., A. GILIEN, AND F. B. BRANDON 1959 Complement fixing antigens in neoplastic tissue extracts. Virology, 7, 348-351.

TEIXEIRA, J. D. AND J. E. SWADEL 1941 Further studies on the serological reactions of infectious myxomatosis. J. Bacteriol., 42, 591-603.

WHEELER, C. E., C. M. CANBY, AND E. P. CAWLEY 1957 Long-term tissue culture of epithelial-like cells from human skin. J. Invest. Dermatol., 29, 383-392.

ZIRKLER, L. A. 1958 Specific tumor antigens. Advances in Cancer Research, V, 291-329.