Reports

Complement Fixation with a Mouse Tumor Virus (S.E. Polyoma)

Abstract. Complement fixation tests with tissue-culture antigens indicated that polyoma virus is serologically unrelated to a wide variety of known viruses; antibody developed uniformly in mice given virus intraperitoneally or intranasally and was found in normal animals of several mouse colonies but not in human beings.

Stewart *et al.* (*1*) have reported the isolation of a virus in tissue culture from several types of mouse neoplasms, including filtrate-induced parotid gland tumors (*2*) and a transplantable leukemia. The virus grown in tissue culture produces a variety of tumors in mice, developing after incubation periods as short as 3 to 4 months. Eddy and her coworkers reported that the agent produces multiple fibrosarcomas and hemangiomas in suckling hamsters (*3*) and cytopathic effects in mouse embryo tissue cultures (*4*). More recently hemagglutination (HA) by the agent and the detection of antibodies by hemagglutination inhibition (HI) tests were reported (*5*). This report describes the development of a complement fixation (CF) test with the polyoma virus, and some of its applications.

Three polyoma strains were studied; two strains (Nos. 3919 and 6069) were received from S. E. Stewart and B. E. Eddy, and the third strain (LID-1) was established in tissue culture in this laboratory. Complement fixation antigens and antisera prepared from these three strains gave reciprocal cross reactions with each other. Since in preliminary tests the LID-1 strain gave higher titers, this strain was used for most of the tests reported here.

Complement fixation antigens were prepared by infection of 8- or 32-oz flask cultures of trypsinized whole mouse embryo; the cultures were prepared by seeding the bottles with 15 or 40 ml, respectively, of a 1:200 dilution of packed cells in a growth medium consisting of 10-percent human serum in Eagle's basal medium. At the time of virus inoculation, the medium was changed to Eagle's basal medium with or without 5-percent horse serum. Fluids were harvested once or twice weekly, and when cytopathic effects were generalized, the tissue was harvested with the supernatant fluid. Individual fluids were tested for antigen against 8 to 16 units of standard mouse antiserum. Pooled antigens titering 1:4 were stored at −60°C and were used unheated. A modified Bengtson procedure was used (*6*).

Standard mouse antiserum was prepared by intraperitoneal inoculation of adult Swiss mice (National Institutes of Health "general purpose" mice) with 0.2 ml of undiluted tissue culture fluid; the mice were bled 3 weeks later. The standard serum titered from 1:320 to 1:640 in CF tests against polyoma virus antigen and was negative at a 1:10 dilution against control antigen prepared from uninfected mouse embryo tissue cultures. This serum titered 1:2560 in HI tests and 1:1280 in tissue culture neutralization tests performed according to the procedure for adenoviruses (*7*), and it prevented development of tumors in suckling hamsters during a 20-day observation period to a titer of 1:1000 (*8*). Control sera, prepared by comparable inoculation of mice with fluid from uninoculated tissue cultures, were negative in CF when tested at a 1:10 dilution against virus and control antigens. Similar tests with immune and control hamster sera gave results essentially identical to those obtained with the mouse sera. Hyperimmune guinea pig sera also reacted specifically with the virus antigens.

Complement fixing antibody developed regularly in newborn and adult Swiss mice given high dilutions of virus intraperitoneally or low dilutions intranasally. Suckling hamsters developed antibody, as well as tumors, after intranasal administration of virus. The susceptibility to infection by the intranasal route suggests that airborne dissemination may be of importance in natural spread of the mouse infection, and that spontaneous cross infection may be a problem in laboratories working with the agent.

Complement fixation antigen, as well as HA activity and infectivity, sedimented into the pellet when tissue-culture fluids were centrifuged at 20,000 rev/min for 2 hours in a Spinco No. 40 rotor (23,360*g*, average); this procedure was of much value for concentrating both the virus and the antigen. As yet, a soluble antigen has not been demonstrated.

In sera of individual mice from naturally infected stocks, CF antibody was invariably accompanied by HI and hamster tumor neutralizing antibodies; however, the CF test was somewhat less sensitive for detection of antibody, since mice with low titer HI and neutralizing antibody were often negative in CF tests.

The LID-1 strain CF antigen did not react with potent antiserums, prepared in mice, guinea pigs, or monkeys, against LCM, mouse salivary gland virus, Coxsackie A types 1 to 19, Coxsackie B types 1 to 5, ECHO types 1 to 20, or poliovirus types 1 to 3. Also, mouse antiserum to the LID-1 strain did not react in CF with the soluble antigens of LCM; influenza A, B, and C; Sendai; hemadsorption virus types 1 and 2; or the adenovirus common CF antigen (*9*).

Young adults and retired breeders of a number of mouse stocks have been surveyed for CF and HI antibody (*10*); at present, antibody has been found, in varying percentages of animals, in certain stocks of C3H, AKR, DBA, RF, C57Br, C58, and Stoli mice. Antibody has not been detected in the stocks of A, STR/N, PRI, CFW/N, BALB/CAnN, BRSUNT/N, or BT mice that have been tested; however, more extensive testing is needed. In positive stocks, antibody was found chiefly in mice over 6 months of age. Although mice with filtrate-induced parotid tumors or leukemias (Gross) were invariably positive, mice with spontaneous leukemias or mice from lines with high rates of spontaneous leukemia were generally negative. Approximately 80 C3H and DBA mice of several sublines carrying the Bittner milk agent which had developed mammary tumors were tested and were found to be consistently negative for CF and HI antibody to polyoma virus; nontumorous mice of these same sublines were also negative. Mice inoculated with the hematopoietic tumor-including virus described by Friend (*11*) did not develop antibody to polyoma virus. These data suggest that polyoma is immunologically distinct from the Bittner and Friend agents, and

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the referneces and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

from the etiologic agents or factors responsible for a number of spontaneous mouse leukemias as well.

It is interesting that C3H/P mice—a strain in which no antibody has so far been found in uninoculated animals—frequently developed CF and HI antibody to the S.E. polyoma virus following inoculation with the C3H leukemia described by Schoolman, Schwartz, and Szanto (*12*); of seven groups of mice receiving tumor extracts and tumor filtrates, at three passage levels, all pooled sera were positive, as were pooled sera of two of three groups inoculated with brain extracts of mice carrying the transplantable tumor. However, of the pooled sera of five groups inoculated with brain filtrates, none of which developed leukemia, only one had antibody. Whether the apparent presence of S.E. polyoma virus in this leukemia bears any relationship to the reported transmissibility of the leukemia by filtrates, as described by Schoolman *et al.*, or represents fortuitous contamination remains to be determined.

Sera from 162 human beings were tested for CF antibody; a 1 : 8 serum dilution was tested against 2 units of S.E. polyoma antigen. The persons tested included 25 healthy adults, 65 healthy children from 1 to 4 years of age, and 72 persons, mostly adults, with solid tumors or leukemia. All tests were completely negative. This absence of reactions indicates that the S.E. polyoma virus does not share common CF antigens with prevalent human viruses such as the adenoviruses, the Coxsackie, ECHO, and polioviruses, the herpes simplex virus, the salivary gland virus, and the human myxoviruses, to which CF antibodies are commonly present in human sera. It also suggests that the polyoma virus does not share CF antigens with hypothetical viruses responsible for the tumors in the patients studied.

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9. Dr. Robert Chanock and Dr. Leon Rosen generously supplied reagents for these CF tests.
10. We are indebted to Dr. Howard B. Andervont, Dr. Ludwik Gross, Dr. George E. Jay, Jr., Dr. Lloyd W. Law, and Samuel M. Poiley for supplying mice for these surveys.
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Fertility in Two Haploids of Solanum tuberosum

Haploid plants ($2n = 24$) of several selections of the common potato, *Solanum tuberosum* L. ($2n = 48$), have been obtained (*1*). These plants were found among seedling progenies of interspecific matings designed to make detection of parthenogenetic individuals relatively easy. Haploids of this species represent tools of potential value for future potato investigations in both applied and theoretical areas of research. The realization of such potentialities is dependent initially upon a reasonable level of fertility in the haploid individuals.

This is a preliminary report (*2*) on the fertility of two haploids of *S. tuberosum*. Fertility has been measured in two ways: (i) by the percentage of good pollen as determined from mature anthers squashed and stained in acetocarmine and (ii) by the results of crosses with other tuber-bearing species.

One haploid, US-W1, from the commercial variety Katahdin, is highly pollen-fertile. From 60 to 75 percent of the pollen grains are plump and stain with acetocarmine (Fig. 1). A limited number of attempts to self-pollinate this individual have not produced any fruit. This may be due to self-incompatibility, which is known to be the usual situation in 24-chromosome, tuber-bearing species of *Solanum* (*3*). However, a larger number of self-pollinations must be attempted before self-incompatibility can be definitely established for the US-W1 haploid.

Pollen from this plant was used in matings with four 24-chromosome, tuber-bearing *Solanum* species [*S. kurtzianum*, P.I. 175434 (formerly *S. macolae*); *S. neohawkesii*, P.I. 210044; *S. phureja*, P.I. 195191, and P.I. 195198 (formerly *S. kesselbrenneri* and *S. rybinii*, respectively); and *S. simplicifolium*, P.I. 218224] (*4*). All these matings resulted in the formation of fruits, each of which contained many viable seeds. This haploid has also been successfully crossed to a selection of *S. tuberosum*.

Meiosis was fairly regular in the US-W1 haploid plant. Twelve bivalents were present at first metaphase in over two-thirds of the microsporocytes examined. A similar regularity in meiosis was observed by Ivanovskaja (*5*) in a haploid of *S. tuberosum* variety Aurora.

Another haploid, US-W3, from breeding selection Minn. 15-2-10-1-2, is only slightly pollen-fertile. The majority of the pollen grains are small, shrunken, and only 5 to 15 percent stain with acetocarmine (Fig. 1). This haploid has failed as a staminate parent in the few crosses attempted to date. The high degree of male sterility was not surprising in view of the cytological findings. Less than 10 percent of the microsporocytes contained 12 bivalents at first metaphase. Univalents were frequent, and the majority of the tetrads contained micronuclei. Multivalents, especially quadrivalents, as well as chromatid bridges, were also present. When this haploid failed to function as a pollen parent, it was used as a pistillate parent. As a female, it was easily hybridized with *S. phureja*, P.I. 225682 (formerly *S. rybinii*), a cultivated 24-chromosome species from South America. Several fruits were obtained, and each fruit contained a large number of seed. Therefore, even though the US-W3 haploid is only slightly pollen-fertile, it is highly functional as a female parent.

The finding of fertility in haploids of the common potato makes their use as future research material appear extremely promising. Genetic studies at the diploid level should now be possible with such materials. The success of this approach would overcome many of the problems inherent in genetic studies at

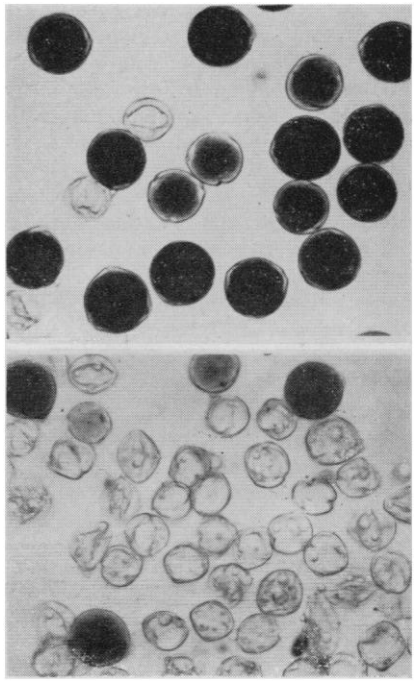

Fig. 1. Pollen grains of US-W1 (top) and US-W3 (bottom).