Table 2. Days necessary to detect bacilli. Fifty-six sputa were examined by each cultural method.

| Cultural method | Positive sputa (No.) | Avg. No. of days for growth |
|---|---|---|
| Medium No. 1 and No. 2 | 30 | 2.5 |
| ATS medium | 23 | 31.1 |

patients suspected of having pulmonary tuberculosis but in whom the sputa were reported as being negative with the Ziehl-Neelsen technique. As parallel and as control cultures, American Trudeau Society medium (ATS) was used as described by Willis and Cummings (*1*).

It is to be noted that 22 of the sputa cultivated were positive in all media employed. Twenty-five sputa were negative in all media used. Eight sputa were positive in medium 1 or 2, and one sputum was positive in ATS medium but not in medium 1 or 2. In the cultures that were positive, growth of acid-fast bacilli was detected in an average of 2.5 days when medium 1 or 2 was employed, while an average of 31.1 days was required for the detection of growth in the ATS medium.

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**Reference**

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19 July 1955

## Desynaptic Pseudoassociations in Secale montanum

Unusual meiotic chromosome associations were observed at diakinesis in an experimental plant of the grass, *Secale montanum* Guss. The plant was obtained by pollinating an Iranian strain of *S. montanum* with x-rayed pollen of the same strain. Observations were limited, for the plant was accidentally destroyed after a single fixation of pollen mother cell materials had been collected. Nevertheless, it is felt that the observations should be recorded.

The plant proved to be heterozygous for an induced reciprocal translocation, but more striking were the peculiar chromosome associations at diakinesis (Fig. 1*A*). These unusual bivalents and translocation configurations involved homologous chromosomes that lay side by side, paired along their entire lengths through late diakinesis, yet connected only at their very ends. The connections, which were simply strands of matrix rather than true chiasmata, were always terminal and connected only corresponding regions of the homologous chromosomes. As is shown in this report, these matrical strands were not effective in maintaining the associations in metaphase I.

Similar meiotic configurations have been designated as "quasibivalents" (*1*), "pseudobivalents" (*2*), and as "*s-s* associations" (*3*). In terminology, I follow Walters, who uses the original term *pseudobivalent* to describe ". . . configurations which have a bivalent-like appearance, but which are not formed by chiasmata" (*2*). However, a more general term, *pseudoassociation,* must be proposed to include the translocation configurations recorded here. Under this terminology, matrical strands that connect the chromosomes are called "pseudochiasmata."

Individual pollen mother cells exhibited both normal and pseudoassociations. An estimated 80 percent of the diakinesis cells contained one or more pseudoassociations. Only six diakinesis cells could be completely analyzed, however. Of the six cells, two showed $5_{II_n} + 1_{IV_n}$, two showed $1_{II_n} + 4_{II_p} + 1_{IV_p}$ (Fig. 1*A*), and two cells showed $5_{II_p} + 1_{IV_n}$ ($n$ is normal association and $p$ is pseudoassociation). No univalents were observed at diakinesis, but one or more was found in 94 percent of the first metaphase cells. Apparently desynapsis of the diakinesis pseudoassociations was the source of metaphase I univalents. At metaphase I, the univalents moved to the poles ahead of chromosomes of normal configurations (Fig. 1*B*). Early movement of univalents toward the poles was indicated by the fact that only four lagging chromosomes were seen in 83 anaphase I cells, although first metaphase cells contained an average of 3.7 univalents per cell. About 20 percent of the anaphase I cells showed unequal chromosome distribution. Further stages of meiosis appeared rather normal. Only two out of 54 anaphase II cells contained laggards, and only 7.8 percent of the microspores contained micronuclei.

That the pseudochiasmata were, indeed, matrix strands rather than true chiasmata was suggested, not only by their appearance, but also by the following considerations. Desynapsis showed the terminal connections to be ineffective in maintaining pseudoassociations through the first metaphase of meiosis. If the connections were true chiasmata, it would be difficult to explain their consistent terminal location. It is unlikely that chiasmata would originate only at the chromosome ends. Neither could the terminal position of the strands be explained as products of terminalization, for the chromosomes did not open out as do normal bivalents.

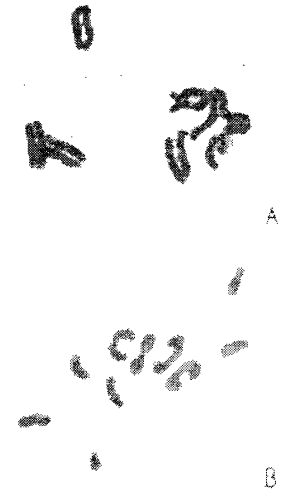


Fig. 1. (*A*) Diakinesis, one normal bivalent, four pseudobivalents, and one pseudotranslocation association of four chromosomes; (*B*) Metaphase I, four bivalents and six univalents.

The pseudoassociations showed interesting deviations from the usual meiotic chromosome behavior. Homologous chromosomes ordinarily synapse along their entire lengths in zygotene and pachytene. In diplotene and diakinesis they separate along most of their lengths but remain connected at chiasmata. Resulting configurations have led to the conclusion that homologs repel each other after pachytene and that they are held together only by chiasmata thereafter (*4*). In diakinesis, the chromosomes of pseudoassociations remained paired along their entire lengths and gave no suggestion of repulsion. Furthermore, the unusual side-by-side association was maintained through late diakinesis without benefit of chiasmata. The terminal strands of matrix could not explain the side-by-side association, for strands were present only at the distal ends of the chromosome arms. Apparently the chromosomes remained paired only through some sort of mutual attraction.

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15 July 1955

## New Type of APC Virus from Epidemic Keratoconjunctivitis

Epidemic keratoconjunctivitis (EKC) is an eye infection with sharply defined clinical characteristics, especially the development of round subepithelial corneal

opacities. Despite multiple and conflicting claims, as reviewed by Cockburn (*1*), "There is at present no virus available that can be regarded with confidence as the etiologic agent of EKC." We have therefore undertaken studies to resolve this problem (*2*).

Conjunctival and corneal scrapings were obtained from a seaman who had just arrived from the Orient and who suffered from typical EKC. The material was treated with antibiotics and inoculated into twice-washed strain HeLa cell cultures (*3*). Maintenance medium (10-percent chick serum in mixture 199) was changed every 4 to 5 days. On the 17th day of incubation, cytopathogenic changes appeared in these cultures, and a transmissible agent was passed in HeLa cultures.

The cytopathogenic effects in HeLa cells resembled those described for APC-RI viruses (*4, 5*). Initially, at the periphery of the cell sheet, cells rounded up and fused into highly refractile clumps. Within the clumps the cells were granular and their outlines were lost. In 2 to 7 days this change spread toward the center of the sheet until the entire culture was involved. The clumps peeled off the glass and floated in the fluid.

The virus was filtrable through Seitz pads and withstood treatment with 30-percent diethyl ether for 18 hours at 4°C. It was not pathogenic by any route of inoculation for suckling or adult mice, guinea pigs, rabbits, or embryonated eggs. A few questionable lesions on chorioallantoic membranes were eventually shown to be nonspecific. The infective titer (TC 50) in stationary HeLa cell cultures at 36°C up to the present 13th passage has not exceeded $10^{-2}$ dilution of culture fluid. The titer was the same both at the onset of cytopathogenic changes and several days later when degeneration was complete. It was not increased by disrupting the infected cells.

The cytopathogenic effect of this virus was neutralized by a homologous antiserum prepared in rabbits (1 to 160), by pooled human gamma globulin (1 to 10), and by serums from patients with EKC, as described in the next paragraph. It was not neutralized by antiserum to herpes simplex or to St. Louis encephalitis virus (*6*) or by monotypic antiserums to types 1 to 7 of the APC group of agents (*7*). Fluid from HeLa cell cultures infected with our virus fixed complement with antiserums to APC viruses, indicating that our virus possessed the soluble antigen of the APC group (*4, 5*).

Serums from patients (*8*) with well-established typical EKC and from individuals with other types of eye diseases were examined for the presence of neutralizing antibodies to our virus. Infected tissue culture fluid (1 to 2) was mixed with an equal amount of inactivated serum diluted with Hank's balanced salt solution. The mixture was incubated 45 minutes at room temperature and then inoculated into twice-washed HeLa cell cultures (0.2 ml per tube) to which maintenance medium (0.8 ml per tube) was added. The tubes were incubated in a stationary position at 36°C and inspected daily for cytopathogenic effects. Readings were taken for at least 4 days after the control tubes (virus and normal rabbit serum) had degenerated. Cytopathogenic effects were graded 0 to ++++. Agreement between tubes containing the same mixture was good. The result was considered to show neutralization when there was a difference of +++ in readings of experimental and control tubes for at least 2 consecutive days. Virus titrations and positive and negative control sera were included in each test.

Table 1. Incidence of neutralizing antibodies to new virus from EKC.

| Disease | Location | Year | Patients | | |
|---|---|---|---|---|---|
| | | | Total No. | No. with neutralizing antibodies* | No. with definite serological evidence of infection (pos./total) |
| Typical EKC | Canada | 1951 | 4 | 4 | |
| | Philadelphia | 1953 | 6 | 6 | |
| | Chicago | 1954 | 9 | 9 | 25/25 |
| | California | 1953–55 | 5 | 5 | |
| | Canada | 1955 | 1 | 1 | |
| Herpetic keratitis | California | 1949–55 | 19 | 1 | 1/19 |
| Uveitis | California | 1954–55 | 10 | 1 | 1/10 |

* Number of patients whose serum neutralized 50 to 100 TC 50 of virus in a dilution of 1 to 10 or greater.

The results are summarized in Table 1. All 25 patients with typical EKC observed in 1951–55 possessed neutralizing antibodies in a serum dilution of 1 to 10 (*9*) or greater from 4 weeks to 3 years after onset of the disease. On the other hand, only two of 29 patients with other types of eye disease had such antibodies. Paired serums from seven patients contained no neutralizing antibodies in a serum dilution of 1 to 5 during the acute illness. All patients with neutralizing antibodies also possessed complement-fixing antibodies to our agent and to RI 67 virus, which merely denoted infection with any of the APC viruses in the recent past.

Follicular conjunctivitis has been observed in patients infected with one of several types of APC viruses, particularly type 3 ("pharyngoconjunctival fever") (*10, 11*), and type 4 (*11*). However, severe keratitis with subepithelial opacities typical of EKC has not been seen in such infections. Furthermore, respiratory symptoms are not associated with the eye disease in epidemics of EKC. Thus EKC does not appear to fit into the pattern of other APC infections (*4, 5, 10, 11*).

The evidence presented in this paper indicates that the virus which we isolated from a typical case of EKC belongs in the group of APC viruses but does not fit a hitherto established type. No claim is made at this time concerning its etiological role in EKC. However, the serological evidence strongly suggests that this virus was regularly associated with typical EKC during 1951–55 in several localities in North America.

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### References and Notes

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6. Kindly supplied by E. L. Lennette.
7. These tests were kindly performed by R. J. Huebner and T. Berge.
8. We are indebted to H. L. Ormsby, M. D. Pearlman, and I. H. Leopold for making such serums available to us.
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26 August 1955