Staff Meeting Bulletin
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University of Minnesota

Experimental Intra-Ocular Virus Infections

Volume XVI | Friday, January 5, 1945 | Number 10

STAFF MEETING BULLETIN
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UNIVERSITY OF MINNESOTA MEDICAL SCHOOL
CALENDAR OF EVENTS
No. 53 January 8 to 13, 1945

Visitors Welcome

Monday, January 8, 1945

9:00 - 10:00 Roentgenology-Medicine Conference; L. G. Rigler, C. J. Watson and Staff; Todd Amphitheater, U. H.

9:00 - 11:00 Obstetrics and Gynecology Conference; J. L. McKelvey and Staff; Interns Quarters, U. H.

12:30 - 1:30 Pathology Seminar; Ethyline Disulphonate. H. H. Brenner; 104 I.A.

Tuesday, January 9

9:00 - 10:00 Roentgenology Pediatrics Conference; L. G. Rigler, I. McQuarrie and Staff; Eustis Amphitheater, U. H.

11:00 - 12:00 Urology Conference; C. D. Creevy and Staff; Main 515, U. H.

12:30 - 1:30 Pathology Conference; Autopsies; Pathology Staff; 104 I. A.

12:30 - 1:30 Physiology-Pharmacology Seminar; Review of the Synapse Theory; Berry Campbell, 214 M. H.

4:30 - 5:30 Obstetrics and Gynecology Conference; J. L. McKelvey and Staff; Station 54, U. H.

4:00 - 5:00 Pediatrics Grand Rounds; I. McQuarrie and Staff; W-205 U. H.

4:30 - 5:30 Ophthalmology Ward Rounds; Erling Hansen and Staff; E-534, U. H.

5:00 - 6:00 Roentgen Diagnosis Conference; T. B. Merner, Solveig Bergh; 515 U. H.

Wednesday, January 10

9:00 - 11:00 Neuropsychiatry Seminar; J. C. McKinley and Staff; Station 60, Lounge, U. H.

11:00 - 12:00 Pathology-Medicine-Surgery Conference; Thrombosis of Right Iliac Artery; E. T. Bell, C. J. Watson, O. H. Wangensteen and Staff; Todd Amphitheater, U. H.

12:30 - 1:30 Pediatrics Seminar; Bone Marrow as a Site for Reception of Infection and Transfusions in Children; Robert Semsch; W-205 U. H.

12:30 - 1:30 Physiological Chemistry Literature Review; Staff; 116 M. H.

4:30 - 5:30 Neurophysiology Seminar; A. Frequency Phenomenon on the Motor Cortex of the Cat; E. Gellhorn; 214 M. H.

Thursday, January 11

9:00 - 10:00 Medicine Case Presentation; C. J. Watson and Staff; Todd Amphitheater, U. H.

4:00 - 5:00 Physiological Pathology of Surgical Diseases; Physiology and Surgery Staffs; Todd Amphitheater, U. H.

4:30 - 5:30 Ophthalmology Ward Rounds; Erling Hansen and Staff; E-534, U. H.

4:30 - 5:30 Roentgenology Seminar; Reviews of Recent Radiological Literature; Staff; M-515 U. H.

Friday, January 12

9:00 - 10:00 Medicine Grand Rounds; C. J. Watson and Staff; Todd Amphitheater, U.H.

10:00 - 12:00 Medicine Ward Rounds; C. J. Watson and Staff; E-214 U. H.

10:30 - 12:30 Otolaryngology Case Studies; L. R. Boies and Staff; Out-Patient Otolaryngology Department, U. H.

11:45 - 1:15 University of Minnesota Hospitals General Staff Meetings; Congenital Atresia of Esophagus; N. L. Leven and B. Lannin; Powell Hall Recreation Room.

1:30 - 2:30 Medicine Case Presentation; C. J. Watson and Staff, Eustis Amphitheater, U. H.

1:00 - 2:30 Dermatology and Syphilology; Presentation of selected cases of the week; Henry E. Michelson and Staff; W-306 U. H.

1:30 - 3:00 Roentgenology-Neurosurgery Conference; H. O. Peterson, W. T. Peyton and Staff; Todd Amphitheater, U. H.

Saturday, January 13

8:00 - 9:00 Surgery Journal Club; O. H. Wangensteen and Staff, M-515 U. H.

9:00 - 10:00 Pediatrics Grand Rounds; I. McQuarrie and Staff, W-205 U. H.

9:15 - 10:30 Surgery-Roentgenology Conference; O. H. Wangensteen, L. G. Rigler and Staff, Todd Amphitheater, U. H.

9:00 - 10:00 Medicine Case Presentation; C. J. Watson and Staff, M-515 U. H.

10:00 - 12:00 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

11:30 - 12:30 Anatomy Seminar; Influence of Adrenal Gland and Hypophysis on the Induction of Allozan Diabetes; A. Kirschbaum; 226 I. A.

II. THE ROLE OF HISTOTROPISM AND OTHER FACTORS IN INTRA-OCULAR VIRUS INFECTIONS

Charles A. Evans
Robert G. Green

A fundamental property of viruses is their obligate parasitism: in spite of numerous attempts by investigators in many laboratories, no one has devised a culture medium free of living cells, that will support the growth of viruses. The fact that some types of investigative work with viruses are more difficult than analogous work with bacteria is true, in most cases, not because viruses are smaller than bacteria but because they must be providdd with a living host in order to multiply.

Progress in the study of viruses has been closely dependent upon the discovery of susceptible animal hosts. Jennerian vaccination was early placed on a practical basis because calves and rabbits were found to be suceptible to the vaccinia virus. The Pasteur treatment for rabies would probably still be unknown, were it not for the high degree of susceptibility of dogs, rabbits, and other animals to the virus of this disease. Influenza was known for generations as a disease seen periodically in epidemics of varying severity. It occupied the attention of some of the most capable bacteriologists during the "golden era" of bacteriology when the causative organisms of so many diseases were discovered. During the great pandemic of 1918 to 1920, millions of people died of the disease and a new group of scientists exerted themselves to the utmost to learn something of its etiology; but they accomplished little except to disprove prevalent ideas of the bacterial etiology of the disease. In 1933 a group of investigators who were accustomed to using ferrets in the study of canine distemper discovered that putting a few drops of throat washings from a person with influenza into the nose of a ferret resulted in a febrile illness that on serial passage in ferrets closely resembled a severe attack of the disease in human beings. Ferret-to-ferret passage increased the virulence of the virus, pneumonia appeared, and it was soon found that as the virulence of the virus increased, the virus acquired pathogenicity for mice. Now the real study of influenza was possible. Using white mice by the thousands (and later fertile eggs in even larger numbers), investigators vied with one another for the discovery of new facts about the pathogenesis, immunology, and epidemiology of the disease. Today our knowledge of influenza is about as complete as it is of any other virus disease, and this knowledge is directly the result of the discovery of susceptible laboratory animals.

Instances of the unfolding of knowledge of certain virus diseases following the discovery of suitable experimental animals could be multiplied. But let us consider the other side of the picture: those virus diseases for which no suitable experimental animal is available, and concerning which our information is accordingly meager. The common cold is clearly in this category. Experiments on human volunteers[1] appear to have established that the infectious agent passes bacteria-proof filters. This fact, together with the regularly negative results that have attended attempts to cultivate it on bacteriologic media establish the viral nature of the agent causing the common cold. Yet we know almst nothing about this disease. Whether carriers occur, the nature of immunity, whether one or numerous viruses are involved--these and many other elementary questions cannot be answered because, up to the present, there is no way of establishing a recognizable infection in laboratory animals. The development of specific vaccines for the virus is obviously impossible without means of propagating the virus.

Much of what has been said of the common cold is equally true of chickenpox. Although it is one of the most common infectious diseases of childhood, our knowledge of the etiologic agent of this disease and of many fundamental facts concerning its transmission, pathogenesis, and immunology is primitive. Experiments with human beings have shown

that the causative agent is a virus, but attempts to carry on detailed studies or to develop specific vaccines are completely blocked by our inability to produce demonstrable infection of laboratory animals.

There is a very considerable list of diseases that appear to be infectious which may be caused by viruses, although definite proof of their viral causation is lacking. In this group one might include such conditions as infectious mononucleosis, some of the atypical pneumonias, dermatitis herpetiformis, Guillain-Barré's disease, and a host of other conditions. Probably some of these should be classified with chickenpox and the common cold as virus diseases for which no susceptible experimental animal has been found.

Still another group of viruses, the study of which proceeds slowly, includes those that will infect only monkeys or other animals difficult to obtain or to handle. The measles virus is infectious for monkeys, in which it usually induces a febrile illness accompanied by a more or less mild rash. Knowledge of the measles virus is still elementary because monkeys are too expensive and too cumbersome to handle to permit extensive and thorough studies with them. The slow progress of information about poliomyelitis is attributable to the same cause.

In this paper, for the sake of brevity, we shall use the term "difficult virus disease" to refer to those diseases with which experimental work is difficult because no experimental animal is available or because the only known susceptible animals cannot be used in large numbers. If a means could be found to produce detectable evidence of infection with any of these viruses in animals available in large numbers, the opportunities for thorough study of the virus and of the infection it causes would be thrown wide open. It is in the attack on this general problem, significant in regard to so many viruses, that the present study of intraocular virus infections had its origin.

Symptomless infections produced by viruses

Many viruses are known to produce symptomless infections in suitable animal species. The salivary-gland disease of guinea pigs is a well-known example.[2] It has been shown that large numbers of guinea pigs in a colony may harbor the virus of this disease in their salivary glands, where its presence can be detected by the inclusion bodies it forms. No local or general signs of the infection are seen in the living animals. Traub[3] and others have described the widespread infection of white mice with the virus of lymphocytic choriomeningitis, a virus which the infected animals harbor for long periods of time without showing any evident ill effects. The well-known virus of mouse encephalomyelitis described by Theiler[4] is primarily resident in the intestinal tract of mice, where it causes no apparent ill effects. Spread to the central nervous system, with resultant symptoms, is seen only in one mouse in several thousand.[5]

In view of the well-known occurrence of symptomless virus infections, it has seemed reasonable to us to consider it possible that some of the human viruses that do not cause visible infection of laboratory animals may actually grow in these animals and produce a silent infection. It was felt that some of these viruses, if inoculated into the eye, might produce sufficient disturbance of ocular tissues so that evidence of the infection could be seen through the cornea. In that case, a "symptomless infection" would become readily apparent and a supposedly resistant animal would be demonstrably susceptible. By this means it was hoped that some of the "difficult virus diseases" could be more readily studied. In considering the likelihood that such silent infections might become "visible" if the virus were put into the eye, it was immediately realized that the kinds of cells available in the eye and the kinds of animals

employed would be of fundamental importance.

Tropisms of viruses

It is well known that parasites are frequently adapted to growth in only certain kinds of animal species. A tapeworm accustomed to the human alimentary canal may be unable to survive in the intestine of a dog. Similarly, the typhoid bacillus from a human being will not cause typhoid fever in a mouse. Viruses have an analogous specificity as to the species of animal they will infect. The rabies virus will attack practically any mammal and many kinds of birds. In contrast, the virus of poliomyelitis is ordinarily infectious only for man and the other primates. This dapstation to particular kinds of animals we have called zootropism.

Viruses also exhibit a high degree of specificity as to the kinds of cells they will infect within a susceptible animal. This so-called histotropism may be illustrated by considering three viruses and the cells they are known to attack within the central nervous system of susceptible animals.

Virus	Cells attacked in CNS of susceptible animals
Fox encephalitis	Vascular endothelial Meningeal mesothelial Ependymal
Herpes simplex	Neurones Neuroglia Fibroblasts of meninges
Rabies	Neurones

Each of these viruses will readily cause a fatal infection of the central nervous system, but the actual site of damage in terms of kinds of cells destroyed differs markedly.

In view of these significant tropisms of viruses, it is apparent that in working with any "difficult virus disease" it is important to employ as many different kinds of animals as possible in searching for a susceptible species; furthermore, it is apparent that the likelihood of success in an attempt to induce a demonstrable intra-ocular infection depends upon what kinds of cells are available in the intra-ocular tissues. A review of the histology and embryology of the eye shows that in the intra-ocular tissues there are many different kinds of cells, probably more distinct kinds of cells than would be accessible to an inoculum placed in any other organ of the body.

Structure of the eye in relation to virus infections

During the development of the embryo, mesodermal tissues are combined with ectodermal structures from both the central nervous system and the skin in the formation of the eye. The lens and the epithelium on the external surface of the cornea are derived from the skin. From the primitive brain, a bilateral outpouching forms and develops into, first, the optic cup and, later, the retina. The pigment epithelium, which is seen as a single layer of cuboidal cells between the choroid and the functional part of the retina, represents the outer wall of the optic cup. Over the ciliary body and the posterior surface of the iris the two layers of the optic cup are represented by a double layer of cells, one pigmented and the other nonpigmented. A curious modification of the cells representing the outer layer occurs in the iris, where they form the muscle fibers of the iris which cause the pupil to expand and contract.

The stroma of the cornea, iris, ciliary body and choroid coat are primarily of mesodermal origin. In the iris and ciliary body, loose connective tissue with numerous small vessels and certain irregularly shaped pigment-bearing cells, the chromatophores, occur. Rather undifferentiated cells of the histiocytic type and plasma cells are commonly encountered. The choroid coat is essentially a highly vascular layer containing numerous pigmented cells.

The anterior chamber is lined anteriorly by a pavement of endothelial-like cells that cover the cornea. These extend over the fibrous arches in the filtration angle and continue over the anterior surface of the iris to the pupil. Aqueous humor is formed by the ciliary body and passes forward through the pupil and out the filtration angle through a thin endothelial filter into the canal of Schlemm and thence to the blood stream.

This brief review indicates the marked diversity of cell types represented in the intra-ocular tissues and indicates that viruses of widely different histotropisms might be expected to grow in the eye.

Experimental intra-ocular infections

Remarkably little work on intra-ocular virus infections is to be found in the literature. A number of investigators have injected neurotropic viruses into the eye in order to study the pathogenesis of the resulting infection of the central nervous system but usually without paying particular attention to the eye. Therefore, the following discussion is presented largely on the basis of original work. As many aspects of the work are currently in progress, the results described are in the nature of a progress report, further studies being necessary in order to complete what has been accomplished so far. Inasmuch as a number of staff members in the clinical departments have generously cooperated in providing materials from patients with illnesses to be studied, it was decided to take this opportunity to give an account of the work in progress in spite of the incompleteness of much of our data.

In our first studies, which were made on intra-ocular infections caused by the virus of fox encephalitis[6,7,8], striking results were obtained. Silent infections were made readily visible, and precise investigations previously impossible became practicable. Further work with other viruses soon showed that with some viruses, unforeseen factors caused the results to be quite different from those anticipated. In elucidating some of the unexpected results, we have obtained information that seems to have a significance in regard to virus infections in general, apart from our immediate problem, and at the same time offers a foundation for the possible application of the intra-ocular method to the study of viruses not readily worked with in the laboratory at present.

Intra-ocular infections with fox encephalitis virus

A virus disease found in foxes and dogs has been described under the name of fox encephalitis.[9] Animals with a natural or an experimentally induced infection develop neurologic disturbances that can be shown to result from numerous hemorrhages in the central nervous system. Although the disease is clinically an encephalitis, the virus is unable to attack neurones directly; it grows in endothelial cells throughout the body. Small hemorrhages are seen in many organs but only in the brain do they give rise to symptoms.

One of the major difficulties in work with this virus in the past has been that the injection of virus intracerebrally or by any other ordinary route would not consistently cause disease in foxes. In some experiments, as few as 10 per cent of a large group of animals inoculated in the brain showed symptoms. Such irregular results made accurate titrations of virus impossible. Similarly, the potency of immune serums or vaccines could not be determined with any degree of precision. Even a rough estimation of the neutralizing power of serum required the use of rather large numbers of foxes and involved considerable expense.

It was believed probable that those foxes that failed to show symptoms after the injection of virus experienced a symptomless infection. From careful studies of the distribution of inclusion bodies in animals dead of encephalitis[10], it was believed that certain intra-ocular tissues would probably be suitable for the growth of this virus. As previously reported,[6] a group of foxes was injected intra-ocularly; virus being

deposited in the anterior chamber of the eye. At daily intervals, eyes were removed for histologic study. A few inclusion bodies were found at 24 hours. The number increased to a maximum at 4 days. At this time the cornea was opaque and obviously thickened and edematous.

In subsequent experiments it was observed that corneal opacity developed regularly after an incubation period of 2 to 4 days and constituted reliable evidence of the growth of virus within the eye. In fact, this opacity came to have the same high degree of significance as an indication of the presence of virus in the inoculum, as death of an animal has in other virus infections. Just as death may occasionally occur from complicating causes, so corneal opacity occasionally developes from causes other than growth of the virus. Therefore, final confirmation was always obtained by finding inclusion bodies in cells of the corneal endothelium. This was easily done by means of a smear.[11]

Using intra-ocular inoculation, we found foxes 100 per cent susceptible to the virus of fox encephalitis unless they had had previous contact with the virus. It was observed that the two eyes of one fox could be used for spearate tests, as virus did not pass over from one eye to the other. The "virus" ordinarily used in previous experiments was a 10 per cent suspension of brain, liver, or spleen from an infected animal. With the intra-ocular method, infection was regularly produced by a 1 to 100,000 dilution of such tissue. This is a most sensitive test for virus.

Mixing specific immune serum with the virus destroyed its infectivity for the eye. Accurate determinations of the amount of neutralizing antibody in the serum were made by employing suitable dilutions of the serum and the virus. The activity of vaccines was readily ascertained by giving vaccinated animals a challenge inoculation of virus in the eye.

Fox encephalitis infections in more resistant species

Inasmuch as intra-ocular inoculation had overcome the difficulty of "silent" infections in foxes, it was thought desirable to re-investigate certain animal species that were considered resistant to the virus of fox encephalitis. Extensive earlier work had established the range of susceptible animal species as including only the foxes, dogs, and wolves.

Twelve rabbits were inoculated intra-ocularly with 2 strains of fox encephalitis virus (6 rabbits with each strain). These animals were killed at intervals of from 1 to 10 days and their eyes were studied microscopically, as well as grossly. No evidence of infection with the virus could be found.

Raccoons had been used in several earlier tests with the virus, a total of 28 being employed in a period of 4 years. Only 1 had died, and its death, at the time, was attributed to incidental causes. Intra-ocular tests showed the eye of the raccoon to be fully susceptible to the virus of fox encephalitis. A typical opacity of the cornea developed at 3 to 5 days and inclusion bodies were abundant. Serial transmission from one raccoon to another was carried through 10 passages. An occasional raccoon died with the symptoms of fox encephalitis after intra-ocular inoculation. Successful infection of the eye of the raccoon constituted the first demonstration that an animal considered resistant to a particular virus may be shown to be fully susceptible if the virus infection occurs in the eye.

In a further study of intra-ocular inoculation of "resistant" animal species, cats have been employed. Results of preliminary experiments indicate that the cells of the eye of the cat are susceptible but to a lower degree than corresponding cells in the eyes of raccoons and foxes. Two kittens were inoculated in the anterior chamber. One of them was killed at 3 days. Both corneas were normal grossly and no inclusions were found on smears. The other, killed at 6 days, showed a slight, questionable

cloudiness of the right cornea and a definite small area of cloudiness near the center of the left cornea. Smears showed a moderate number of inclusions in the right eye and fewer in the left. Two adult cats failed to show opacity of the cornea after intraocular inoculation, and no inclusions were found in smears from their eyes.

These results must be confirmed on additional animals before the difference in susceptibility of old and young cats can be considered conclusive. However, it is evident that ocular tissues of the cat exhibit a lower degree of susceptibility than ocular tissues of either the raccoon or the fox.

Quantitative differences in susceptibility to fox encephalitis

It is of interest to note that the apparent quantitative difference in susceptibility can be ascribed to specific cell types. Sections of numerous eyes of foxes and raccoons have shown that the fox encephalitis virus attacks specifically the endothelium (or mesothelium) of the cornea, filtration angle, and iris, with further spread to the stroma of the iris and ciliary body. Opacity of the cornea is the obvious manifestation of edema; and the edema, in turn, is due to disruption of the endothelium on the posterior surface. The normal transparency of the cornea is the direct result of a state of relative dehydration maintained by the presence of hypertonic fluids bathing both surfaces of the cornea. As has been clearly shown by Cogan and Kinsey[12] both the external epithelium and the internal endothelium form semi-permeable membranes through which the tear fluid and the aqueous humor draw water from the cornea stroma, into which it diffuses from the limbus. The obvious result to be expected when virus destroys endothelial cells is accumulation of fluid in the corneal stroma and resulting opacity.

It would seem from the results of preliminary experiments that the maintenance of transparency of the cornea in the infected eye of a cat is due to a low degree of susceptibility of the corneal endothelium to the fox encephalitis virus. It is well known that animal species may differ quantitatively in susceptibility to a virus. That there can be a similar quantitative difference in susceptibility of particular kinds of cells has not been so clearly shown. It appears that from a comparison of the observed infections in cats, raccoons, and foxes, we are beginning to get an insight into the actual basis of quantitative differences in susceptibility to a virus.

Histrotropism as shown by intraocular infection

Detailed studies of the distribution of inclusion bodies in the eyes of foxes have shown cells of the following types to be infected: fibroblasts in the stroma of the cornea near the limbus and in the stroma of the iris, ciliary body, and choroid; endothelial cells lining the anterior chamber; large mononuclear cells, probably histiocytes, in the filtration angle; vascular endothelium in the iris, ciliary body, and occasionally the retina or choroid; and, in at least one instance, a chromatophore in the iris. In no eye have ganglion cells, bipolar cells, rod or cone cells shown inclusion bodies. Neither have the supporting cells of the visual retina been involved. This parallels the observation that in the central nervous system neither nerve cells nor neuroglia are infected. It is of interest that ependymal cells lining the walls of ventricles contain numerous inclusions if virus has been inoculated into the cerebrospinal fluid,[13] but that ependymal cells covering the chorpid plexus are quite different---they never contain inclusions. In the eye, the cuboidal epithelium covering the ciliary body and internal surface of the iris are embryologically and morphologically somewhat analogous to ependymal cells. In only one instance has an inclusion body been found in cells of this type, a non-pigmented epithelial cell of the ciliary body. Also, in only a single instance has an inclusion been found in a cell of the pigmented epithelium of the visual retina. No inclusions have been seen in

smooth-muscle cells of the iris. Clearly, these various cells possess a relatively high resistance to the fox encephalitis virus.

Intra-ocular infections with feline agranulocytosis virus

One of the most common and severe infectious diseases of cats is known as feline agranulocytosis, or cat distemper. In various clinical forms of the disease, the predominant features may include enteritis with severe diarrhea, catarrhal inflammation of the upper respiratory tract with crusted exudate around the nose and eyes, and progressive weakness and prostration. A profound leukopenia is a characteristic finding. The causative virus produces intranuclear acidophilic inclusion bodies much like those of herpes simplex. The presence of these inclusions shows that the virus infects several kinds of cells, including epithelium of the intestinal mucosa and certain cells in lymph follicles of the intestinal wall, mesenteric lymph nodes, and liver.

In view of the high virulence of this virus for cats and the kinds of cells known to be attacked by it, it was anticipated that virus inoculated into the eye would readily find susceptible cells and would induce a highly destructive process that could be watched through the cornea. Six adult cats were inoculated in the eye with a strain of the virus received from Dr. J. T. Syverton. The needle was inserted through the sclera and the inoculum was deposted in contact with the choroid, retina, ciliary body, and iris, as well as in the aqueous humor and vitreous humor. One cat was immune, presumably from previous exposure. The other 5 became severely ill on the fourth or fifth day after inoculation. Three died on the fifth and sixth days and 1 was killed in a morbund state on the sixth day. One recovered after a severe illness.

Even though the infections in these cats were severe, no significant intraocular changes could be detested in daily examinations. At the height of the illness, Dr. Tracht of the Department of Ophthalmology examined the cats and agreed that no significant abnormalities could be found. Fundoscopic examinations were not performed inasmuch as we are interested primarily in infections visible on ordinary inspection.

Eyes from 4 of the cats were available for microscopic studies. Inclusions were few in these eyes in spite of the obvious virulence of the virus employed. A careful field-by-field search of sections from the eyes of a cat with more inclusions than the rest, showed that a considerable number of types of cells in the iris, ciliary body, and retina were infected. These included fibroblasts in the retina and ciliary body, retinal epithelium on the iris, pigmented epithelium on the ciliary body and over the visual part of the retina, and ganglion cells and bipolar cells in the visual retina. Our expectation that numerous intra-ocular tissues would be susceptible to the virus was fulfilled, but in spite of the fertile ground for growth, and the high virulence of the virus employed, the intraocular infection was strikingly limited in extent. It appeared that infection had spread very little from the cells infected by the original inoculum. This limitation of spread was in marked contrast to the situation in the rest of the animal body, where rapidly fatal infection had occurred.

Two explanations of the limited involvement of the intraocular tissues might be postulated. First, the observed results might be expected if the cells in the eye of the cat were susceptible in low degree, as the feline endothelial cells are to the fox encephalitis virus. This seems unlikely in view of the multiplicity of kinds of susceptible cells (ganglion cells, cuboidal epithelium, fibroblasts). It was noted that in certain areas where the retina had been split, apparently by the inoculum, foci of inclusions involving a number of adjacent cells were present. The impression was gained from these studies that the virus of feline agranulocytosis, although virulent for individual cells, lacked the capacity to invade those cells when the normal continuity of the tissue

was intact. It appeared that the normal surfaces of the retina, ciliary body, and iris constituted an effective barrier to invasion of the virus.

The results obtained with feline agranulocytosis showed that a virus fully capable of infecting cells of intra-ocular tissues may not induce a visible and experimentally useful infection in the eye. The reasons for this apparently anomalous situation are unknown. A possible explanation has been suggested, but more work needs to be done before definite conclusions can be drawn. The practical implications, insofar as work with "difficult virus diseases" is concerned, are obvious.

Intra-ocular infections with the virus of herpes simplex

The virus of herpes simplex, which is well known as the cause of fever blisters and cold sores in man, is readily transmitted to mice and to rabbits by cutaneous or intracerebral injection. The characteristic intranuclear inclusions make it easy to determine which cells the virus attacks. Extensive studies by Goodpasture and Teague[14] and others have shown that these include epithelial cells of the skin and mucous membrane, neurones and neuroglia cells of the central nervous system and peripheral ganglia, hepatic cord cells, transitional epithelium of the kidney pelvis, fibroblasts, and a number of other kinds of cells. In view of this broad histotropism and inasmuch as the rabbit is highly susceptible to the virus, it was anticipated that intra-ocular inoculation might result in a violent infection readily apparent through the cornea. This has not been found to be the case.

In a series of experiments that is continuing, 10 rabbits have been given intraocular injections of herpes virus in various doses and under a variety of circumstances. The results, while still incomplete, indicate that intraocular inoculation is not a particularly sensitive test for small amounts of virus. In one experiment, more than 20 times the amount of virus that was infectious for a mouse by brain inoculation was injected into the eyes of a rabbit (anterior chamber and vitreous) and failed to cause infection detectable by either gross or microscopic examination. Even when large doses of virus are injected, the ocular reaction is not at all conspicuous. Hyperemia and edema of the iris with mild to moderate circumcorneal injection are the only changes usually seen. In some eyes a mild degree of haziness of the cornea develops, but this never approaches the opacity seen in fox encephalitis. Fundoscopic studies have not been done. Encephalitis commonly develops, as would be expected, from the known neurotropism of the virus.

In spite of the relatively mild changes seen in infected eyes, there has been found an abundance of susceptible cells in the eye. The following kinds of cells have shown inclusion bodies: ganglion cells, bipolar cells, and rod and cone cells of the visual retina; pitmented epithelium, fibroblasts, and muscle cells of the ciliary body; epithelium, radial and circular muscle cells, pigmented epithelium and fibroblasts of the iris; macrophages in the filtration angle or in an area of infiltration in the retina, and endothelium and epithelium of the cornea. With such a comprehensive list of susceptible cells in the eye, it has seemed somewhat baffling that the intra-ocular infection should be anything but violent. One sees in sections that certain foci of virus infection occur with necrosis of cells, development of inclusions and infiltration of polymorphonuclears. It appears that in the case of herpes simplex, as in intra-ocular infection with the virus of feline agranulocytosis, the virus readily infects cells with which it comes in contact at the time of inoculation, but that subsequent spread is limited. The mechanism of this apparent limitation of spread is unknown but may well be the same in the case of the two viruses.

In spite of the apparent "limitation of spread," one may create considerable areas of damage in the ciliary body and iris by using a large dose of virus. We have been impressed by the relatively severe damage that must be done in these

areas before conspicuous changes become apparent in the eye as seen on ordinary inspection. This limits the usefulness of intra-ocular inoculations in the study of certain "difficult" viruses.

It should be remarked that the infiltration of polymorphonuclear leukocytes is often considerable in intra-ocular herpetic infection but is seldom sufficient to cause a noticeable turbidity of the aqueous humor.

Intra-ocular infections with the virus of ornithosis

The ornithosis virus is well known as a cause of atypical pneumonia in man and of a severe disease of many kinds of birds. It is one of the largest known viruses, being readily visible with the microscope, and produces characteristic basophilic inclusion bodies in the cytoplasm of infected cells. The large size of the virus, the characteristic morphology and staining properties of the inclusions, and a number of other factors provide evidence of a rather close taxonomic relationship of this virus with a number of other viruses, including those that cause lymphogranuloma venereum, trachoma, inclusion conjunctivitis, a pneumonia of cats, and a pneumonia of mice. It is our intention to study intra-ocular infection with several of these viruses. As yet we have employed only the virus of ornithosis* a strain of which was supplied to us by Dr. F. R. Heilman, who isolated it from a case of human atypical pneumonia. This virus was maintained by brain-to-brain passage in mice and was an unusually virulent strain for mice. A 10 per cent suspension of infected mouse brain in Ringer's solution was injected into the anterior chamber of 1 eye of each of 3 rabbits. The needle was inserted through the sclera, behind the iris to the pupil, where the tip passed forward into the anterior chamber. The 3 animals were killed at 3, 5, and 8 days.

Because of the highly infectious nature of the ornithosis virus, close inspection of the eye was made only on the day an animal was killed. However, the presence of a moderate amount of mucopurulent exudate was noted at 24 hours and 48 hours after inoculation. (Cultures of the inoculum on blood agar plates were sterile.) Gross inspection of the rabbit killed at 72 hours showed that the inoculated eye had undergone considerable damage. Moderate conjunctival exudate was present. The entire cornea was clouded and thickened with edema. Smears of aqueous humor and of corneal endothelium showed a moderate number of polymorphonuclear leukocytes and smaller numbers of large mononuclear cells that we have called macrophages. Typical inclusion bodies were found in the macrophages. The endothelial cells appeared normal on smears. In spite of careful search, no inclusion bodies were found in these cells. The normal appearance of the corneal endothelium was surprising in view of the edema and opacity of the cornea observed grossly. In the second rabbit, killed at 5 days, the findings were essentially the same as in the rabbit killed at 3 days. At 8 days, the eye of the last rabbit showed similar changes of an even more severe degree. The cornea was thickened and edematous and the entire eye appeared to have enlarged, increasing the diameter of the cornea. Adhesions between the iris and cornea had developed.

Sections of the eye removed at 3 days yielded findings quite different from those produced by any of the viruses previously employed. The purulent exudate seen externally had a counterpart in a marked infiltration of leukocytes into the anterior chamber, where they accumulated in the meshes of fibrin. Marked infiltration of the ciliary body and of the substantia propria of the cornea and sclera at the limbus were observed. The iris was edematous and

*With the discovery that psittacosis is a disease affecting many kinds of birds, some zoologically quite distinct from parrots, K. F. Meyer[15] suggested that the term ornithosis be employed unless one refers specifically to the disease as it occurs in psittacine birds. This term has gained increasingly widespread usage.

congested but almost no cellular infiltration was present.

Inclusions were common in macrophages in the anterior chamber and filtration angle, and a smaller number were found in the substantia propria of the cornea, where virus was presumably carried by cells migrating from the filtration angle. A typical inclusion was also seen in a fibroblast. None were seen in the iris. The endothelium of the cornea appeared resistant to the virus. At one point where a small defect in the cornea was present, an endothelial cell at the margin of the defect contained an inclusion body, and two large cells that seemed to be moving from the endothelial layer out into the anterior chamber also had inclusions.

Two unexpected facts appeared evident from a study of these sections. First, although there was an abundance of virus in the aqueous humor, it did not invade the substance of the iris. The histiocytes and fibroblasts of the stroma of the iris are doubtless susceptible to the ornithosis virus, in view of the presence of inclusions in similar cells in the filtration angle and cornea. But the delicate partition formed by endothelial cells on the surface of the iris must have constituted a barrier, preventing invasion of the stroma of the iris by the virus.

The second unexpected finding was the remarkably healthy appearance of corneal endothelium as seen in smears, as well as in sections. From what is known of corneal physiology, it seems certain that the severe edema resulting from intraocular ornithotic infection is due to interference with the functioning of the endothelium as a semi-permeable membrane. The small amount of cellular infiltration and the few inclusions in the corneal stroma appeared wholly insufficient to cause the edema on the basis of direct involvement. It appeared that inasmuch as the actual infection of corneal endothelium as shown by inclusion bodies was negligible, the damage might be due to a toxic effect from virus in aqueous humor. This seems more probable in view of the recent reports of Rake and his associates on a definite toxic property of the viruses of lymphogranuloma venereum, mouse pneumonia, and meningopneumonitis.[16]

The toxic effect of influenza virus in the rabbit eye

The effect of intra-ocular inoculation of influenza virus has been studied in a series of experiments conducted principally in the Influenza Laboratory of the Minnesota Department of Health by Dr. E. R. Rickard in collaboration with one of us (C.A.E.). A preliminary report of results is now in press.[17]

The PR8 strain of influenza type A virus and the Lee strain of type B virus have been employed. These viruses are maintained by passage through chick embryos. The blood-free allantoic fluid from infected embryos, which is used as inoculum, contains a large amount of virus, as is shown by the fact that there are about 100 million infective doses of virus for eggs in 1 cc. of allantoic fluid (50 per cent end point is $10^{-7.8}$ for Lee and 10^{-8} for PR8). These viruses are also relatively virulent for mice, as the 50 per cent mortality end point for the Lee strain is $10^{-5.7}$ and for the PR8 strain is $10^{-5.5}$

Injection of 0.2 cc. of infected allantoic fluid into the anterior chamber of the rabbit eye regularly leads to a marked corneal opacity and edema which are usually readily observed at 72 hours and reach a maximum by the fourth day. During the first 24 or 48 hours, while the cornea is still transparent enough to permit clear visualization, a marked redness of the iris, sometimes accompanied by prominent radial folds of the iris, is noted. The corneal opacity may persist for weeks or, particularly if Lee virus or diluted PR8 virus has been used, may disappear after several days or a week or more.

The decidedly positive results obtained with these influenza viruses were particularly interesting, inasmuch as rabbits have been found by most investigators to be resistant to influenza viruses. It was considered necessary to

determine whether the corneal reaction was due to the virus itself or to some nonspecific toxic substance in the allantoic fluid. Such a toxic substance might conceivably be discharged by cells disintegrating because of infection with the virus, or might be an excretory product of the chick embryo normally present in allantoic fluid. The latter possibility was ruled out by showing that allantoic fluid from uninfected chick embryos caused no opacity of the cornea when injected into the anterior chamber of the rabbit eye. A series of neutralization tests showed that the ocular reaction was due to the virus itself or to some closely related substance. In these tests, serum from ferrets that had recovered from infection with the PR8 or Lee strains of virus was mixed with virus prior to inoculation into the rabbit eye. In every instance neutralization was specific and complete. PR8 virus mixed with homologous antiserum was inocuous to the eye, but PR8 virus mixed with antiserum specific for Lee virus retained its full effect on the cornea. Similarly, specific results were obtained with the Lee virus. Therefore, it was evident that the factor causing corneal opacity must be either the virus itself or some substance closely associated with it in the ferret, as well as in the chick embryo.

Further studies have shown that a variety of factors that will reduce the infective titer of the virus, simultaneously destroy its power to induce corneal opacity. Heating at 56°C., filtration through a Seitz filter, and treatment with dilute formalin have been used in the experiments of this sort. We next sought to determine whether virus grew in the rabbit eye. It was considered possible that, as in the case of raccoons injected with the virus of fox encephalitis, rabbits given influenza virus by ordinary routes underwent a symptomless infection, whereas if the infection was induced in the eye, it became readily visible. Results of experiments soon showed that this is not the case.

Rabbits were injected in the eyes with the usual dose of allantoic fluid rich in virus, and then at intervals an animal was killed and the aqueous humor and tissues of the eye were tested for the presence of virus by the injection of embryonated eggs. By diluting the inocula in graded amounts and determining the highest dilution that would infect eggs, the amount of virus remaining in the eye was estimated. Six eggs were inoculated in each test. Results showed that even at 8 hours the virus had disappeared from the aqueous humor and that within 24 hours, or less, the amount of virus that could be accounted for in the tissues indicated that a 99.99 per cent reduction had occurred. The virus in the tissues remained at about the same low level (end point 10^{-2} or 10^{-3}) for 2 or 3 days. Tests of 2 eyes removed on the fourth day showed that the virus had almost completely disappeared, as only 1 of 12 eggs injected with a 10 per cent suspension of tissues was infected.

The results of this experiment showed that the virus did not multiply in the rabbit eye. The effect on the cornea must therefore be considered a toxic action, similar to the toxic activity that was postulated, on much less complete evidence, in the case of the ornithosis virus. In line with the toxic rather than the infectious nature of the observed corneal reaction were the results of experiments in which serial dilutions of virus were injected into the eyes. A 10-fold dilution of infective allantoic fluid usually produced a typical corneal reaction, but a 100-fold filution was inactive in the rabbit eye. These findings contrast with those for mice and chick embryos, in which very high dilutions are infective.

While we were preparing these data for publication, there appeared a short description, by Henle and Henle,[18] of the toxic effect of influenza virus injected into mice intracranially in large amounts. Although the virus does not multiply, it causes the death of mice with neurological symptoms. Presumably, this toxicity described by the Henles is analogous to that which we have observed in the rabbit eye. It is interesting to note that toxicity is not

so specific as infectivity, for an animal (the rabbit) resistant to infection is fully susceptible to the toxic effect.

Nature of toxic effect

That the toxic property of influenza virus, demonstrable in the rabbit eye, may have some significance in the pathogenicity of the virus is an obvious point for speculation. It therefore becomes of interest to inquire a little further into the details of this "toxic" effect. As already pointed out, the normal "dehydration" of the cornea is maintained in part by the endothelial lining which serves as a semipermeable membrane through which water flows from the cornea into the hypertonic aqueous humor. Continuity of this membrane might be broken either by destruction of endothelial cells or by disturbance of the intercellular substance. Inasmuch as in ornithosis and also in influenza there appears to be no marked destruction of endothelial cells, we have come to suspect that the "toxicity" may be based on a destruction or alteration of the intercellular substance rather than a noxious effect on the cell proper. This, however, is no more than a speculation that will be tested by experiments now under way.

Intrao-cular infections with miscellaneous viruses

Hog cholera is a severe and frequently fatal virus disease of swine characterized by high fever, loss of weight, and hemorrhages into the skin. Ths virus is present in large amounts in the blood and spleen of infected animals. In several experiments we have inoculated young pigs in the anterior chamber of the eye or in the vitreous humor with hog cholera virus. A few animals have developed a partial and usually transient cloudiness of the cornea, but no consistent abnormality due to the virus has been noted. In all instances, a severe generalized infection of the type usually seen in hog cholera has developed. Unfortunately, inclusion bodies are not produced by the virus and it is therefore impossible to determine precisely which cells are infected. It seems almost certain, however, that some of the intraocular cells must support the growth of the hog cholera virus. Probably in this disease, as in feline agranulocytosis, limited growth of virus occurs in the eye but the resulting damage is insufficient to induce changes easily detected by inspection.

The virus of canine distemper, a common disease of dogs, produces characteristic inclusion bodies that may be found in a great variety of cells, including epithelial cells of the urinary tract, bronchial tree, biliary, salivary, and pancreatic ducts, and skin; secretory cells of the adrenal medulla; neurones, neuroglia, and ependymal cells of the central nervous system; and lymphoid or reticular cells of lymph nodes. In view of this broad histotropism, it seems more than probable that a number of different kinds of susceptible cells must also be present in the eye.

In several experiments we have employed a strain of distemper virus that was passed through ferrets continuously for several years. During that time the virus became of such low virulence for dogs and foxes that it is commonly injected as a live-virus vaccine. Intra-ocular inoculation of this virus into dogs and foxes regularly fails to induce detectable signs of infection in the eye.

The virus of poliomyelitis has been injected into the eyes of 10 monkeys, usually into the anterior chamber, in several experiments. In no instance has a significant intra-ocular reaction due to the virus been noted. However, intra-ocular injection has proved to be an excellent means of infecting the nervous system. The first sign of infection is usually a ptosis of the lids, which may become so marked as to completely obstruct the vision of the animal. Nervousness and progressive paralysis of the extremities occur as they do with infection by any other method. Burnet, Jackson, and Robertson[18] have employed intra-ocular inoculation in preference to intracerebral infections in conducting neutralization tests with the poliomyelitis virus.

Discussion

Experience with a variety of viruses has shown that a number of unexpected factors must be considered in any attempt to utilize intra-ocular infection as a means of studying viruses. These may be briefly summarized as follows:

1. The eye contains a great variety of cells which are susceptible to a proportionately large number of viruses.

2. Whether or not a readily visible (and therfore useful) reaction occurs after intra-ocular inoculation of a virus depends not only on how many intra-ocular cells are susceptible to infection but also on how many of those cells actually become infected. As was clearly shown in the case of feline agranulocytosis, and as was apprently true in herpes simplex, ornithosis, hog cholera, and canine distemper, a virus may be markedly restricted and infect only a relatively few of the susceptible cells in an inoculated eye. It appears that in the eye there may be remarkable inhibition of spread of some viruses. Intact surfaces of tissues within the eye seem to be particularly resistant and even the delicate and almost invisible endothelial covering of the iris appeared to block the invasion of a highly virulent strain of ornithosis virus.

3. Moderately extensive infection of cells of the iris and ciliary body fail to cause a readily apparent change that can be seen on ordinary inspection of an eye. This was evident in the studies with the virus of herpes simplex.

4. The only really useful type of ocular change that we have encountered is corneal opacity. Such opacity may result from either of the following two mechanisms:

   a. Infection and destruction of corneal endothelium as seen in the case of fox encephalitis.

   b. A toxic effect of the virus itself or of something closely associated with it. The corneal opacity that follows inoculation of influenza virus into the eye of a rabbit is purely a result of toxicity, for no growth of the virus occurs. Intra-ocular inoculation of a strain of ornithosis virus resulted in severe infection, with inclusion bodies in the anterior chamber and elsewhere, and the formation of considerable cellular exudate. Surprisingly, the endothelial cells appeared normal in all 3 rabbits studied, although all 3 animals had corneal edema and opacity. In this instance the effect on the cornea was evidently also one of toxicity.

Several observations incidental to the main problem are of sufficient interest to merit comment. It is generally recognized that the type of cellular exudate seen in bacterial infections is in some degree a characteristic of the kind of bacteria causing the infection. There are, for example, "pyogenic cocci." From the results of our studies of intra-ocular infections, somewhat the same would appear to be true in the case of viruses. In the rabbit eye, the viruses of ornithosis and herpes simplex appeared to be pyogenic, in that they called forth a precominantly polymorphonuclear exudate. On the other hand, the viruses of fox encephalitis, feline agranulocytosis, influenza, and canine distemper, did not stimulate the formation of such exudates. Whether the "pyogenic" response to the herpes and ornithosis viruses was a reaction to the viruses themselves or to the products of cellular degeneration cannot be stated with certainty, and probably the same is true in the case of the "pyogenic cocci."

Differences in degree of susceptibility of animals to infectious agents are well known, but the factors responsible for the differences have not been clarified. In work with fox encephalitis, we have been able to make a beginning in analyzing these factors. In the following

table the pertinent facts have been tabulated:

	Intracerebral inoculation	Endothelial cells of cornea
Foxes	Susceptible	Completely susceptible
Raccoons	Resistant	Completely susceptible
Cats	Resistant	Lower degree of susceptibility
Rabbits	Resistant	Completely resistant

If one were to test 10 or 12 animals of each species by intracerebral inoculation of fox encephalitis virus, the results would probably be as indicated, although in our experience, one out of 28 raccoons came down with the disease. Two facts are clear from this table; first, a "resistant" animal species may have highly susceptible cells; second, the susceptibility of cells to a virus may vary quantitatively from one animal to another.

Intra-ocular involvement is an unusual complication of human virus infections. This is true in spite of the facts that (1) many viruses circulate in the blood stream, (2) the eye is a highly vascular organ, and (3) cells of many types, susceptible to many viruses, are present in the eye. From the observations recorded in this paper, one might speculate that a significant factor in the rarity of intra-ocular complications is an exceptionally great resistance to spread of virus within the eye. Whereas a neurotropic virus that succeeds in passing the blood-brain barrier appears to have little further resistance to overcome in spreading extensively within the central nervous system, a virus that succeeds in passing the "blood-eye barrier" is apparently confronted by formidable defense mechanisms that limit its development. We have also been led to wonder whether small foci of virus infection in some of the intra-ocular tissues might not be rather easily overlooked, and whether they occur more commonly than is generally believed.

Conclusions

1. Intra-ocular infection has proved useful in eliminating symptomless infections in experimental work with the virus of fox encephalitis. Infection regularly causes corneal opacity.

2. Injection of influenza virus into the anterior chamber of the rabbit eye induces corneal opacity as a result of a toxic effect without concomitant infection.[17]

3. A strain of ornithosis virus injected into the eyes of 3 rabbits caused an intra-ocular infection with considerable cellular exudate and a corneal opacity that apparently was due to a toxic effect.

4. Intra-ocular infection with the virus of herpes simplex is not a sensitive method of detecting the virus. Large doses of virus will cause considerable infection, as can be demonstrated by microscopic studies, but the changes visible grossly are usually limited to congestion and edema of the iris. This type of infection is not so clear-cut as corneal opacity and would be of limited usefulness in virus studies.

5. When the viruses of feline agranulocytosis and hog cholera were inoculated into the eyes of susceptible animals, intra-ocular infection was not sufficiently severe to cause recognizable changes in the eye, even though violent and, in many cases, fatal generalized infection ensued.

6. Intra-ocular inoculation promises to be of value in working with viruses that: (a) cause a destructive infection of endothelial cells; (b) have toxic properties such as have been demonstrated in the case of the influenza and ornithosis viruses; and (c) cause very extensive infections of other intra-ocular tissues.

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III. GOSSIP

The holiday season opened Sunday, Dec. 18 at our house. Celebration of Christmas and the New Year automatically becomes the great event in our lives as we attempt to relive the joys of childhood. Sunday, December 18, we went to the great Christmas party for the children in the hall. It is an old fashioned neighborhood affair at our little church which duplicates celebrations the whole country over. The children are there bright and early, occupying the front seats. There is an air of expectancy and excitement. After many delays the show starts. The modern version is a showing of movie comics and travelogs, followed by the ever popular magician show in which the children applaud but little so intent are they in catching the magician in his mistakes. Then the event proceeds to higher levels, for now the children participate with songs, recitations and group entertainment. At this point there is apparently something wrong with arrangements for Santa Claus who was supposed to have arrived is late, and everyone is thoroughly alarmed over what may have taken place. But - the door opens and in bounds the jolly old fellow with his bells ringing like mad. His pack is filled with candy and popcorn balls. Our children and the neighbors' children receive not only a single allotment, but in many instances two and three allotments for Santa Claus has an abundance in his old pack, bringing out more than he realized he had.... On Monday we visit our older daughter's school to see the play called "Our Lady of the Market". A lovely, colorful affair built around the old Christmas story in which the part of Our Lady is taken by a beautiful young girl who remains immobile throughout the entire performance. About her the older and younger "women" of the market place celebrate Christmas in the manner of Mexico. Tuesday, to the hospital party where all are invited to partake of an abundance of cakes, cookies and coffee and what have you. At this time of the year there is no distinction between staff members. On Wednesday, the highlight of the season as far as the hospital is concerned for the Traffic Club comes to trim the trees. For over 20 years this organization of shippers has represented Santa Claus in our institution. Their able little helper, Dorothy Jones, and many other members of our organization are here to help. The ornaments are brought out to decorate the trees, a few trees are lighted, and then we go to the lounge in the nurses' home for our get-to-gether with these good people. This is only the beginning of the many pleasant reminders of their good will during the Christmas season. They will come again to give a Christmas party for the children. Santa Claus is always represented by the same man. Each child will receive the presents he requests. All the patients young and old will have a Christmas remembrance. The nurses will join them later in the singing of carols, and even though the sick and attendants must remain here during the holiday season, they are not forgotten....On Thursday to Duluth, where I have luncheon with our good friend and supporter, E. L. Tuohy. Rotary day and the daughters of Rotary members come to celebrate with their fathers. An elaborate program again made brighter by the presence of Santa Claus. This time he is a different kind of Santa Claus. A Santa Claus who makes speeches, who sings songs, a Santa Claus with an unmistakable Scandinavian accent, a Sants Claus who thought that this was a grand and glorious opportunity to put us right as far as the affairs of the world were concerned. A most unusual Santa Claus, but one most characteristic of this most unusual place, Duluth....On Friday to annual Surgical party, another highlight of the Christmas season. For many years the Surgical group have gathered together with their friends to celebrate in a most elaborate way. On Saturday to the radio to tell of the greatest gift of science to mankind during the past year. Undoubtedly penicillin deserves this reward and so it is selected for the subject. On many other Christmas-times, in fact, every year since 1928, this has been an annual affair with me, and each year it has been easy to select some great contribution which has been a boon to our people. Later to speak on Medicine in the News on Mental Hygiene. Separation from home and loved ones, however, precludes a complete sense of well-being. And so we learn as we must in all of our great problems of life, to make the necessary adjustments as to time, place, etc. ... HAPPY NEW YEAR.