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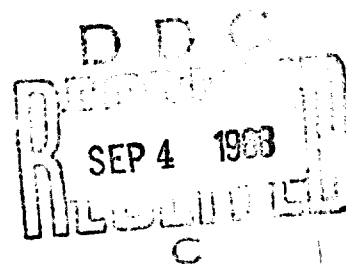
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EXPERIMENTAL EYE INDUCED INFECTION OF THE CONJUNCTIVA
WITH NEWCASTLE DISEASE VIRUS IN HUMAN VOLUNTEERS

Following is a translation of an article by
S. Lutte and G. Pino of the University of Sassari
Eye Clinic in the Italian-language Bollettino
d'Oculistica (Bulletin of Ophthalmology), Vol.
30, 1951, pages 553-562.

Newcastle disease, also known as pseudo-plague or
aviary pneumoencephalitis, is an infectious disease of fowl
attacking those animals' nervous systems and their respira-
tory and gastro-intestinal tracts.

Described for the first time by Kraneveld in the
Dutch East Indies in 1926, it was identified by Doyle in
1927 as the pathogenic agent during an epidemic in the vi-
cinity of Newcastle-on-Tyne, England; for this reason its
name has been taken from this city and it is still commonly
referred to as such.

The agent causing this disease is a virus of medium
size (Newcastle disease virus or NDV as referred to by Eng-
lish-language writers) varying from 80 to 120 millimicrons
(as determined by both filtration and the electron micro-
scope).

The viral elements appear with an irregularly spheri-
cal shape if studied in allantoic fluid under the electron
microscope, while they take on a filamentous shape (recall-
ing that of spermatozoa) when examined in physiological so-
lution.

They remain active when preserved in 50 per cent gly-
cerine, when deep-frozen in a refrigerator at -70°C. and
when freeze-dried. They are inactivated by heat (at 60°C.
for 30 minutes or at 35°C. for 45 minutes), by methylene
blue, by ultraviolet rays and by formalin (1:5000 solution).

It is relatively easy to grow the virus in chicken
embryos; it is found in highest concentration in the allan-

toic fluid.

In addition to being pathogenic for many species of fowl, both wild and domesticated (chickens, turkeys, ducks, geese, pigeons, guinea-hens, pheasants, sparrows, crows, parrots, etc.), as well as for mice when inoculated by way of their nasal passages; it is not transmitted from one mouse to another, though.

It agglutinates the blood of chickens, man and several other animal species (Burnet, Chu, Bozzo and Pine).

Infection by NDV induces the appearance of specific antibodies in the circulation. Newcastle disease is widespread over a vast area; it has been observed in Europe, Asia, Africa, Australia, the United States, Canada, etc.

Its pathogenic agent, in addition to being of microbiological and veterinary interest, has also taken on importance in the field of human pathology and, more specifically, in the field of ophthalmology, because it has shown itself able to induce conjunctivitis.

The first observation was made in 1943 by Burnet who noted a case of conjunctivitis due to infection in the laboratory. Since then many cases of the like have been described (Shimkin 1945, Yaton 1946, Anderson 1946, Radnot 1947, Kujumgiev 1948, Freymann and Bang 1948, Ingalls and Mahoney, Garcia Miranda 1949, Jacotot et al. 1950, Keeney and Hunter 1950).

The only case known to date in Italy was the recent one described by Borsello and Mantovani.

Altogether more than forty or so observations have been reported by various authors, most of them being of a sporadic nature and less often grouped in small epidemics (17 infected subjects in Yaton's report, 5 in that of Garcia Miranda, 3 in that of Lépine, Atanasiu and Gareau).

In all cases the contagion has been demonstrated or has been assumed to have arisen from infected fowls or from laboratory material.

The set of symptoms was sufficiently typical and showed almost invariable features. As a rule the disease appeared on one side only. The period of incubation lasted about 24 hours. In only a few cases did it last as long as two or three days (Yaton, Anderson, Ingalls-Mahoney).

Subjective symptoms comprised itching, lacrimation and sometimes dryness of the conjunctiva, the feeling of having a foreign body in the eye and moderate hypersensitivity to light.

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Objectively one found a more or less marked edema of the eyelids in some cases extending to take in the cheek. The conjunctiva of the eyelids appeared highly hyperemic, slightly edematous and sometimes hemorrhage was noted, though generally only to a moderate degree (Shimkin, Borsello and Mantovani). Moderate hypertrophy of the optic disks was also noted as well as the development of follicles, particularly in the lower fornix conjunctivae. In some cases the plica semilunaris conjunctivae was greatly swollen and hyperemic (Borsello and Mantovani); the bulbar conjunctiva showed different reactions in different cases, being affected by conjunctivitis in the case reported by Borsello and Mantovani, while moderately hyperemic in the case reported by Keeney and Hunter.

In only one case it was noted that the cornea had become involved in the form of small, pinpoint epithelial infiltrations some of which could be stained with fluorescein (Keeney and Hunter).

These made their appearance on the second day of the illness and had all disappeared by the tenth day.

The secretion was of a catarrhal type, being copious in some cases as in that reported by Borsello and Mantovani, while in other cases it was rather scarce and consisted primarily of tears.

An examination of conjunctival smears brought out the presence of leukocytes (chiefly neutrophilic and lymphocytes), some blood and fibrin. Keeney and Hunter claim to have observed epithelial inclusion bodies in conjunctival scrapings.

The conjunctival form was almost always accompanied by affection of the preauricular ganglion and often by moderate illness, chills and fever.

With respect to treatment of NDV in humans, one finds that Sheehan first unsuccessfully used antistine (proprietary name for a synthetic antihistamine compound, 2-phenyl-benzyl-aminomethyl-imidazoline hydrochloride) on a chicken raiser suffering from conjunctivitis as a result of NDV infection, but found that local administration of aureomycin brought about improvement in the clinical picture 24 hours after treatment.

Another chicken grower, a neighbor of the first, also affected by the same disease, is said to have quickly shown improvement upon administration of aureomycin.

Keeney and Hunter, in consideration of the fact that aureomycin was found to be effective in treating conjuncti-

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vitis caused by inclusion bodies and acute follicular conjunctivitis of unknown origin but resembling the type described by Béal, attempted to treat NDV conjunctivitis with an 0.5 per cent aureomycin borate collirium instilled into the right-hand eye every hour and a penicillin collirium in a concentration of 5,000 Oxford units per ml. in the left-hand eye. No improvement was noted and active virus was isolated from both eyes after four days of this treatment.

Lépine, Atanasiu and Gareau also experienced negative results using aureomycin.

RESEARCH CARRIED OUT BY THE AUTHORS

Inoculation of human conjunctiva with NDV

Since not all of the cases reported up to now have been accompanied by an accurate clinical description and others present no results of blood tests and other laboratory tests, the only means of being sure (in many cases the diagnosis was based exclusively on the existence or the assumption of contagion) we thought it worthy of interest to reproduce this new disease experimentally in human subjects in an attempt to give a complete clinical and laboratory picture of the disease.

Original inoculation -- To this end four volunteer subjects (one of the authors and four students of medicine) were inoculated in the conjunctival sac of the left eye with a vial of allantoic fluid from chicken embryos infected with NDV (strain Na of the Istituto Sieroterapico Italiano; Italian Serotherapeutical Institute).

The period of incubation varied from 12 hours (three subjects) to 24 hours (two subjects).

The subjects began to feel itching of the conjunctiva and an uncomfortable sensation of a foreign body in the affected eye. These symptoms continued to increase in intensity, accompanied during the following two or three days by lacrimation and hypersensitivity to light (photophobia). In spite of the lacrimation the subjects experienced a sensation of dryness, more intense on awaking in the morning.

These symptoms slowly declined until they disappeared after the first week of the illness.

Initial objective examination disclosed moderate edema of the eyelids, particularly of the lower lid. In two subjects there was only a minimal degree of edema.

At first a marked hyperemia of a red-wine color was noted at the conjunctiva of the eyelids and at the fornix.

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Subsequently they appeared to be slightly velvety with an increase of the so-called papillary bodies. In two cases occasional petechiae were noted, particularly along the free edge. A moderate superficial congestion of blood vessels was noted in the bulbar conjunctiva. This became more intense and wine-red in the neighborhood of the conjunctival folds; concomitant with this there was considerable edema and hyperemia of the caruncle and the plica semilunaris (fold of mucous membrane at the inner canthus of the eye).

These phenomena increased during the first two or three days of the disease and then slowly declined.

Between the end of the first day of the disease and the beginning of the second many follicles appeared, particularly in the lower conjunctival fornix where they appeared somewhat large and stood out, being arranged to form a small wreath.

These follicles became increasingly evident as the congestive phenomena gradually subsided. It should be pointed out that in some subjects the congestive phenomena were more outstanding than the presence of follicles.

In one of the subjects the congestive phenomena were at an absolute minimum while the development of follicles was very outstanding.

As to the color of the follicles, they were bright pink in those subjects with marked congestive phenomena and paler and in some cases even tending towards pearl gray in those subjects experiencing a lesser degree of conjunctival hyperemia.

In all cases the conjunctival secretion was rather small in amount and of the lacrimal type. The symptoms were always accompanied by swelling of the preauricular ganglion to the size and shape of a small pea, having a hard, springy consistency and moderately painful to pressure. No corneal lesions were observed in any of the cases even though the subjects were examined daily under a slit lamp upon having been treated in advance with fluorescein.

On the second day of the illness two subjects experienced general indisposition, chills and fever which in no case ever exceeded 37.5° and in one subject the fever lasted eight hours, while in the other it persisted 24 hours. The illness lasted a total of three to four weeks. The edema of the eyelids disappeared in all subjects during the first week while the acute congestion of the conjunctiva could be said to have completely subsided during

the second week. The last to disappear were the follicles and the hypertrophy of the preauricular ganglion which persisted in two subjects until the third week of the illness while in the others it could still be felt, even though very much reduced, up to the fourth week.

In all the subjects the infection remained strictly localized to the one eye originally attacked by the virus.

Experiments with reinoculation -- Upon reaching complete cure on the 25th day from the original inoculation, two of the volunteers infected both conjunctival sacs with a vial of the same strain of NDV virus.

After a period of 12 to 24 hours a conjunctivitis of the usual type already described appeared in both eyes, but much more markedly in the right-hand eye, that is, in the eye inoculated for the first time, while in the other, which had already been subjected to such an infection once, the symptoms, though similar to those in the right eye, were much more diminished.

One subject's preauricular ganglion was considerably affected while the other's was affected only very slightly.

Laboratory analyses

Blood tests -- Blood counts failed to show any significant data on the red blood cell count side of the ledger.

Obvious changes took place in almost all cases (four out of five subjects) both quantitative and qualitative, with respect to the white blood cells in the form of leukopenia (a dearth of leukocytes) generally appearing during the second day of the disease with relative lymphomonocytosis (See Table 1).

Investigations of kidney and liver function -- Urinalysis and liver function tests showed constantly normal results in all patients.

Microscopic analysis of conjunctival secretion smears -- Every day during the first week of illness and every other day during the second and third weeks smears were taken of the conjunctival secretions. They showed that the secretion consisted of mucus, a few lymphocytes, a very few neutrophils and epithelial cells which had flaked off. There was a complete absence of any microbes.

Microscopic examination of conjunctival scrapings -- Scrapings taken from the conjunctival epithelium were suitably stained with Giemsa's stain to look for any inclusion bodies which may have been present but the results in

	11-4-1950	13-4-1950	18-4-1950	6-5-1950
V. A.	G. B. 7600 N. 74, L. 25 M. 1	G. B. 5200 N. 41, L. 51 M. 6, E. 2	G. B. 6880 N. 51, L. 46 M. 2, E. 1	— — —
B. C.	G. B. 6000 N. 70, L. 28 M. 2	G. B. 6000 N. 57, L. 37 M. 4, E. 2	G. B. 3100 N. 55, L. 39 M. 5, E. 1	— — —
S. R.	G. B. 5000 N. 55, L. 41 M. 2, E. 2	G. B. 4100 N. 50, L. 45 M. 4, E. 1	G. B. 6000 N. 58, L. 37 M. 2, E. 3	G. B. 5800 N. 50, L. 47 M. 2, E. 1
A. C.	G. B. 6400 N. 75, L. 21 M. 1, E. 2, B. 1	G. B. 4800 N. 62, L. 32 M. 5, E. 1	G. B. 5100 N. 58, L. 37 M. 4, E. 1	G. B. 5800 N. 70, L. 25 M. 3, E. 2
J. J.	G. B. 6400 N. 67, L. 27 M. 4, E. 2	G. B. 5100 N. 49, L. 47 M. 1, E. 3	— — —	— — —

Table 1 - White cell blood counts of five subjects infected with Newcastle disease virus by the conjunctival route on 11-4-1950 (same date as first column) and on subsequent days.

Legend

First column - initials of subjects inoculated.

G.B. = white cell blood count

N = per cent neutrophils

L = per cent lymphocytes

M = per cent monocytes

E = per cent eosinophils

B = per cent basophils

all cases were completely negative.

Bacteriological examination of the conjunctival secretions -- Seeding of the conjunctival secretion in various culture media (ordinary agar, blood-agar, Loeffler) gave negative results in all cases.

Isolation of virus from conjunctival washings -- Each day for a period of six days from the start of the illness a sample of conjunctival washings was obtained from the infected eye or eyes of our subjects.

The washings thus collected and kept in a refrigerator at -20°C. were then inoculated by the intra-allantoic route (0.25 ml.) after having first added penicillin and streptomycin, into eggs containing chicken embryos during their 7th to 11th days of development. The eggs thus inoculated were further incubated at a controlled temperature of 35°C. for four to six days; both positive results from hemagglutination tests and findings of the typical p. cho-

logical anatomic lesions were used as indices of the development of the virus. In cases which proved negative as many as six consecutive runs were made from one egg to the next; during positive cases the virus was generally discovered in moderate quantity on the second run and sometimes even on the first.

Results of the tests carried out by the authors are shown in Table 2; it shows that isolation of the virus from the washings of the conjunctiva gives positive results on only the first two or three days of the disease. In tests carried out later than this tests turned out negative in all cases, even when eye symptoms were at their peak. The treatment with aureomycin given in only one of our cases does not seem to prevent the virus from developing in the tissues of the conjunctiva (positive isolation of the virus up to the second day of illness as in the eye not treated with aureomycin).

Patient	Eye infected	Day of illness on which conjunctival washings sample was taken					
		I*	II*	III*	IV*	V*	VI*
S. R.	O.S.	+	+	+	-	-	-
B. G.	O.S.	+	+	+	-	-	-
U. S.	O.S.	+	+	+	-	-	-
P. G.	O.S.	+	+	+	-	-	-
V. A.	O.S.	+	+	+	-	-	-
	O.D.						
	O.D. (trattato con aureomicina)						

Table 2 - Isolation of virus from the conjunctival washings.

Legend

O.S. Left-hand eye

O.D. Right-hand eye

(trattato con aureomicina) = treated with aureomycin

Immunological investigations -- In order to study the immunizational response to conjunctival infection with NDV in addition to the reinfection tests mentioned above the authors also sought for the appearance of antibodies specific to the virus in question in the blood of the subjects inoculated.

Blood was taken two times from our subjects from the cubital vein in the forearm for this purpose, the first sample being taken before the infection and the second sixteen days afterwards; in each of the blood samples thus obtained (heated for half an hour at 56°C.) an assay was

to determine the presence of antibodies specific for the strain of NDV used by us, using the hemagglutination inhibiting test according to the Hirst method as modified by Sal.

Results of these tests are reported in summary form in Table 3 and confirm the possibility of a serological response to NDV infection of the conjunctiva; in three cases, in fact, there was a significant increase in hemagglutination inhibiting antibodies, though not attaining very high levels. It must be emphasized at once, though, that this response was not constant; it was completely absent in two of our patients, even though they had experienced an identical infection of the conjunctiva both as to kind and as to intensity, to that experienced by the others whose serological response showed positive.

Blood antibody assay

Patient	Before infection	15 days after start of infection
U. S.	< 10*	80
P. C.	10	10
S. R.	< 10	40
E. C.	10	80
V. A.	< 10	10

Table 3 - Assay of NDV hemagglutination inhibiting antibodies in serum.

Note: The figures shown in the table are the reciprocal of the maximum dilution of each serum capable of neutralizing four hemagglutinating units of NDV.

Therapeutic tests -- A one per cent collirium of aureomycin was instilled in a subject infected by NDV in the manner described in the foregoing.

The treatment was started six hours after inoculation and continued for a week (8 instillations per day). The patient did not appear to show any improvement or to obtain any benefit from it and the virus was isolated in its active state for two days from the conjunctival sac.

Remarks and Conclusions

By experimental inoculation with NDV one can produce an acute catarrhal type of conjunctivitis in human subjects consistently, with no excessively accentuated subjective symptoms and with objective signs characterized by edema of the eyelids of a moderate intensity, hyperemia and congestion of the conjunctiva and particularly of the carun-

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cle and the plica semilunaris with above all the development of follicles.

It should be pointed out that we have never in any of our experiments found any cases as intense as those described by Borsello and Mantovani. This could depend on the virulence of the strain used by them or on the organic reaction of the individual. In addition, in all cases it has been found that the preauricular ganglion has been involved with two cases of moderate general indisposition, chills and transitory fever of moderate entity.

In conclusion, a form of conjunctivitis is induced which reminds one very much of Béal's type of follicular conjunctivitis.

Keeney and Hunter have attempted to distinguish between the two diseases, insisting on the greater development of follicles or the absence of corneal lesions in the Béal form of the disease.

These arguments do not seem to us to be too valid by way of differential diagnosis. In fact, the development of follicles can also differ in different cases of NDV conjunctivitis with the individual. We take pleasure in pointing out in this respect one of our cases already mentioned where the follicular hypertrophy was very outstanding and, one could say, was the only thing which stood out in the whole spectrum of symptoms.

As to corneal lesions, one should note that they were not only entirely absent in all our experiments but that as far as we are able to find out they have been observed exclusively in the case reported by Keeney and Hunter.

The presence of small hemorrhages in certain cases of NDV conjunctivitis (which would not occur in Béal's conjunctivitis) does not seem to us to be sufficient to be of any use for differential diagnosis.

The only fact which would remain would be that according to Thygeson's investigations of the Béal form of conjunctivitis, there has been no success in bringing out the existence of any viral pathological agent. It would seem to us, though, that this fact cannot be taken as absolute. Béal's follicular conjunctivitis is, in fact, only a specific set of clinical symptoms and can be looked upon even as simply a conjunctival syndrome wherein various causative agents can play a part, among which the Newcastle disease virus may be only one.

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Nor do collateral laboratory tests seem at the present time to be able to give decisive data in every case to clear up such uncertainties of interpretation as cannot be settled by clinical study. Leaving out of consideration the changes in the white blood cell picture, too atypical and not specific enough to make possible any use as a diagnostic aid as well as the cytoplasmic inclusions described by Keeney and Hunter which seem for the moment to constitute findings in exceptional cases such as we have failed to note in any of the cases studied by us, there remain for consideration on one hand the isolation of the virus from conjunctival secretion and on the other the appearance of specific antibodies in the circulation.

There can be no question of the positiveness of the proof given by either of the two tests. We would only mention the fact, though, that one can assay either the hemagglutination inhibiting antibodies or the virus neutralizing antibodies in carrying out serological tests; in both cases one must use sera heated for half an hour at 56°C. in order to destroy those aspecific inhibiting or neutralizing factors which are present even in normal sera and which could bring about highly untrustworthy results.

In this respect one need only recall what happened to Howitt et al who failed to adhere to this simple requirement in the neutralization tests conducted by them using sera; they believed they had identified a group of human neurological infections attributable to NDV and were mistaken; Howitt, himself, in a subsequent paper, recognizing the error he had made, called attention to the need for destroying the aspecific factor by means of heat, being as it was susceptible to this form of treatment, before going ahead with the assay of neutralizing antibodies.

The thing which interests us though is to emphasize the significance of the negative tests brought out by our research; one cannot definitely state on the basis of failure to isolate the virus or failure to demonstrate the presence of specific antibodies in the circulation or, rather, failure to show any significant increase in their level, that there has or has not been any eye infection by NDV. The transitory nature of the positive indications given by cultures of the conjunctival secretion in chicken embryos which, as we have seen, can cease to appear on the second day of the illness and the possibility of an absence of any serological indication (in two patients out of five in the cases studied) even when a clearly documented and proven infection has definitely occurred constitute in our opinion

factors which undoubtedly render uncertain every differential diagnosis based on the failure to demonstrate the presence of the causative agent.

SUMMARY

Newcastle disease virus conjunctivitis was induced experimentally in five volunteers (1 doctor and 4 students). The general response produced a picture substantially similar to that associated with Beal's acute follicular conjunctivitis accompanied in some cases by slight general symptoms. Laboratory analysis showed leukopenia with relative lymphocytosis and irregular appearance of specific antibodies in the circulation. No NDV could be isolated from the conjunctival discharge after the third day from onset of the disease. No cytoplasmic inclusions were observed in the cells of the conjunctival epithelium. Reinoculation tests, previously infected and recovered conjunctival mucosa proved less receptive. Treatment with aureomycin drops produced no effect whatever.

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