

## **Conjunctivitis of the Domestic Fowl and an Associated Rickettsia-like Organism in the Conjunctival Epithelium.**

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OCULAR ROUP is a well-known complaint of fowls. Every poultryman is familiar with the reddened conjunctiva, the purulent discharge, the cheesy exudate lying deep between the nictitating membrane and eye, the inflammation of the periorbital tissues, the keratitis and even the panophthalmia. In nearly all cases there are some other signs of roup, such as rhinitis, stomatitis, pharyngitis, laryngitis and subcutaneous abscesses over the cranium.

Known causes of "ocular roup" are avitaminosis A, fowl-pox, infectious laryngotracheitis, fowl cholera, streptococcosis and infections produced by *Haemophilus gallinarum* and the cocco-bacilliform bodies of Nelson. The tubercle bacillus will, very rarely, also evoke a conjunctivitis.

From time to time farmers have written to this Institute describing outbreaks of roup that affected only the eyes. We were, however, unable to obtain specimens for examination before November, 1938, when chicks were brought here from Devon in the Transvaal. The two birds, a month old, were undersized on account of the poor ration they received. One had a mild purulent conjunctivitis of both eyes and the lids tended to stick together. The other had a bilateral sero-purulent conjunctivitis, and the tissues round the eyes were moderately swollen; the cornea was only very slightly turbid. The conjunctival epithelial cells of all the affected eyes contained Rickettsia-like organisms in varying numbers. Smears of the conjunctival scrapings were also full of pus cells. From the second chick an unsuccessful attempt was made to infect two adult hens and three cockerels of three months of age. This failure will be referred to later.

In January, 1939, a live White Leghorn pullet, five months old, was received from Frankfort in the Orange Free State. The right eye was normal, except that the iris was lighter in colour than it should have been. These "light eyes" are common in fowls and have nothing to do with conjunctivitis. The left cornea was opaque

and the conjunctiva was moderately inflamed. Conjunctival smears from the left eye contained epithelial cells holding Rickettsia-like organisms. Three weeks later infected cells were very difficult to find. When this fowl first arrived, unsuccessful attempts were made to transmit the disease to two other adult fowls, by instilling conjunctival scrapings containing Rickettsias into their conjunctival sacs. Likewise I failed to infect the bird's normal right eye from its left eye. Six calves proved to be refractory. Finally, the faeces of the diseased fowl were instilled into the eyes of normal birds with negative results.

A third outbreak was encountered in Johannesburg in June, 1939. The subject for examination was a nine-months-old White Leghorn pullet. The left eye was normal. The right cornea was slightly opaque and there was a meagre conjunctival discharge. A smear of the conjunctival scrapings was stained with Giemsa, and only a very few epithelial cells were found containing the organisms. Lymphocytes and monocytes, however, were numerous and there was no doubt that the fowl had almost recovered from the infection.

#### THE EXPERIMENTAL DISEASE.

It is well known that the great majority of workers on the disease called specific ophthalmia or infectious keratitis of cattle have failed to effect its transmission to experimental animals. On the other hand, direct transmission of ophthalmia from sheep to sheep is relatively easy. Our persistent failures to infect fowls were puzzling. Then I decided to hold small pledgets of cotton wool in forceps and rub them firmly over the infected epithelium. Immediately afterwards, the pledgets were rubbed even more firmly over the conjunctival epithelium of normal birds. Success was immediate. Now we can infect 25 per cent. to 75 per cent. of the experimental fowls. It is interesting to note that one eye may become infected, but not the other. Perhaps a similar technique will permit positive results to be obtained in cattle.

The mode of natural transmission is unknown. All the diseased fowls were kept in two special houses on the poultry plant at Onderstepoort. The condition had never been found in our own flocks. Within six months of commencing our experiments, odd cases of the disease were discovered in different intensive poultry houses. There was never any direct contact between the birds and, as we have shown, artificial transmission is not easy. We can only think that flies spread the infection.

The source of the material for our successful experiments was a cockerel that contracted the disease spontaneously, when put in a cage near that occupied by a naturally infected bird. The incubation period may be as short as two days, and the Rickettsias appear simultaneously with the clinical symptoms. No bird ever developed conjunctivitis without showing the organisms. The usual incubation period is three or four days; sometimes the period is even seven or eight days. Although both eyes are infected simultaneously, one may show symptoms a day or two before the other.

The first symptom is a slight swelling or puffiness of the lower eyelid and this can best be observed from the outside. Photophobia is seen early, if the disease is at all severe. The conjunctiva becomes reddened and swollen, particularly inside the lower lid. There may be a very slight muco-purulent conjunctival exudate, and a smear of this shows it to be composed mainly of pus cells. Only occasionally do the lids tend to stick together.

If the disease is severe, the cornea becomes opaque six to seven days after infection. In another two or three days, blood vessels can be seen growing towards the centre of the cornea from its periphery. If the keratitis takes its usual course and resolves within seven to ten days of its appearance, the blood vessels disappear. In very rare cases, however, the pannus may persist for three or four months; the only fowl that showed this, had the affected cornea opaque and bulging; the opacity was due to scarring. Panophthalmia is a very unusual complication.

The acute inflammation of the conjunctiva generally subsides within a week to a fortnight. It sometimes persists for a month. After this the eye gradually returns to normal, the corneal opacity and any pannus disappearing, and all swelling leaving the lids. But, should the eye have been very bad, there is a not uncommon sequel; as the inflammation improves, the nictitating membrane becomes pulled, up to a third of the way across the eyeball. This defect is of a semi-permanent nature.

Occasionally secondary organisms, such as cocci, invade the diseased conjunctiva, and then the lids stick together more easily and there is a gummy discharge from the eye and a lump of cheesy exudate may form under the nictitating membrane.

Symptoms of a systemic nature may be noted. If a female develops the disease severely in both eyes, she remains mopy and dejected for two to four weeks. But in spite of this, the hens show considerable tenacity and go on feeding. In similar circumstances males do very poorly. Frequently they give up the struggle and refuse to eat, and consequently very soon develop blackish combs and yellowish-green diarrhoea and become emaciated. Most of these males die, even as early as 24 days after infection, or they have to be killed. Severe systemic disturbances were not seen when the disease was confined to one eye.

The inflammatory changes are restricted to the structures of the eye. For this reason, rhinitis, sinusitis and pharyngitis do not develop. Even the periorbital tissues show comparatively little swelling. There is no "roupy" smell.

#### ATTEMPTS AT TREATMENT.

Owing to the satisfactory results given by Uleron in the treatment of heartwater, which is due to *Rickettsia ruminantium*, this was the first drug tried..

## CONJUNCTIVITIS IN THE DOMESTIC FOWL.

Three White Leghorn chickens, infected six days previously, got half a Uleron tablet (1 tablet=0.5 gram) *per os* daily for four days. The birds were eight weeks old. Three White Leghorn chickens and another two chickens, all 12 weeks old, that had been infected 13 and 7 days previously, respectively, received half a Uleron tablet each *per os* night and morning. Altogether the birds were dosed 8 times. Five chickens, 13 weeks old, infected 5 days previously, got one Uleron tablet each *per os*, morning and evening. Altogether these birds were dosed 8 times.

Adequate controls were kept. No evidence could be obtained to show that Uleron, in the doses given, could influence the course of the disease. No toxic symptoms were noticed.

Three White Leghorn pullets and another five pullets, infected 9 and 5 days previously, respectively, were each given *per os*, 0.25 gram M. and B. 693; next day the same dose was given in the morning and repeated in the evening and, the day after this again, a single dose was administered in the morning. Thus each pullet got 4 gram of the drug. No toxic symptoms were noted. M. and B. 693 had no adverse effect on the parasites.

One White Leghorn pullet, another 4 pullets and another 7 pullets, infected 17, 13 and 7 days previously, respectively, were dosed *per os*, morning and evening, on two consecutive days with 0.25 gram sulphonamide; on the third morning they were dosed again. Each bird thus received 1.25 grams sulphonamide. No toxic symptoms were observed. The results were again disappointing; apparently the drug has no effect on the Rickettsias.

Uleron, M. and B. 693, and sulphonamide failed to cure the birds in the doses given. Possibly larger doses over a longer period might succeed, but prolonged treatment is not economical on farms.

## IMMUNITY.

Generally speaking, fowls over 18 months old were very resistant to the means of infection employed. Sometimes, however, the disease was produced, and for this reason it is more probable that the method of infection was at fault than that the eyes were not susceptible.

Both eyes of an adult hen resisted infection on two occasions, three months apart. The same applied to a pullet, only the interval was six weeks. But it is dangerous to draw conclusions, for another pullet, infected in both eyes, developed the disease only in the right eye; 22 days after the original infected material was applied to the conjunctiva, symptoms appeared in the left eye. As this left eye had been under observation, it is practically certain that it developed the disease naturally, after experimental infection had failed. Still another pullet was infected unsuccessfully in the left eye, and sixteen days later the disease occurred naturally in the right.

Frequently, when both eyes were infected, the disease, together with the parasites, developed only in one.

Two birds that had the disease badly and recovered, were reinfected after eight and nine months respectively. Even in the eyes that had been so severely affected before, more Rickettsias and clinical symptoms developed. A similar experiment, performed on three other birds, indicated that they were possibly immune, as there was no reappearance of parasites or lesions.

The only conclusions that may be drawn from the above observations are: (1) a recovered eye may be reinfected eight months later, (2) resistance of the eye to experimental infection does not necessarily imply immunity, and (3) infection in one eye does not necessarily lead to the establishment of immunity in the other.

An unsuccessful attempt was made to infect two adult Pekin ducks; the significance of this is questionable.

As far as we know, the natural disease and the attendant parasites are confined to the domestic fowl.

#### DESCRIPTION OF THE RICKETTSIA.

The parasites have an intracytoplasmic habitat in the conjunctival epithelial cells. The nuclei of the host cells are never invaded. A cell may be packed with granules. The organisms may be scattered loosely throughout the cytoplasm or be arranged in groups, resembling colonies. These colonies are not attracted to the nuclei, as similar inclusions are in trachoma and blennorrhoea neonatorum of man. If anything, the parasites tend to gravitate to the edges of the cell (v. Fig. 3); the margins thus often show up very clearly when numerous infected cells lie side by side. Sometimes a colony occupies half of the cytoplasm, leaving the other half free.

It is customary to find a number of clean cells together, and then a number of infected cells next to each other. This suggests that a cell is invaded by Rickettsias from the margin of an adjoining cell.

The organisms are very pleomorphic and approximately  $0.3\mu$  to  $1.5\mu$  in size. Some are rods showing bipolar staining; others are plain rods. Then there are coccal forms, triangular forms, comma-shaped forms, whole rings and broken rings. Not infrequently the granules resemble boomerangs. In any one cell, the parasites conform more or less to a type: they may, for instance, be coccal or bacillary in nature.

With Giemsa, most of the organisms stain a light reddish-blue colour. Some of the largest coccal forms may be light blue, resembling initial bodies, and some of the smallest may stain like chromatin, resembling elementary bodies. But there is no sharp division into initial and elementary bodies, such as we find in trachoma and psittacosis.

The granules are Gram-negative and non-acid-fast. With Pinkerton's technique, the organisms stain blue and not red, a fact that excludes this Rickettsia from the group causing typhus and Rocky Mountain spotted fever.

In staining by this method, a smear is made and dried in air, but not fixed. 0.5 Per cent. basic fuchsin in water is applied for three minutes. The fuchsin stain is run off, and the smear decolourized with 0.5 per cent. citric acid in water for a few seconds. The smear is washed in water, and counter-stained with 1 per cent. methylene blue.

It cannot be said definitely that the parasites damage the host cells, but this must be presumed seeing that the conjunctiva becomes inflamed.

The above description of the organisms seems to be sufficient justification for regarding them as a species of *Rickettsia*. Consequently the name, *Rickettsia conjunctivae galli*, is provisionally proposed for the parasite.

#### RELATIONSHIP OF THE ORGANISMS TO THE DISEASE.

In other articles it has been demonstrated how a certain type of conjunctivitis of the sheep, ox, goat, and pig is invariably associated with the presence of *Rickettsia*-like organisms in the conjunctival epithelial cells. In this paper a similar state of affairs is shown to exist where the domestic fowl is concerned.

With the onset of conjunctivitis in the fowl, conjunctival smears show numerous *Rickettsias* and pus cells. When healing commences, the parasites disappear and the pus cells are replaced by lymphocytes and some monocytes. Thus, in all essential respects, the disease is on a par with that in the other species of domestic animals.

Corresponding to the course of the acute phase of the disease, the parasites may persist for as long as a month. Occasionally they are present for only a day or two. If the parasites are never present in the eye in sufficient numbers to make their detection rapid, no clinical signs of the disease will be apparent. The converse is not equally true, for smears of clinically normal eyes may contain numerous *Rickettsias*. But large numbers of parasites are accompanied by abnormal numbers of pus cells and the eye is thus diseased, even if not clinically so. Generally speaking, large numbers of organisms are associated with obvious clinical lesions.

Unless the conjunctival scrapings are contaminated by exudate adhering to the edges of the lids, remarkably few bacteria will be found. Generally they are absent, and the only visible organisms are the *Rickettsias*. This observation applies also to ophthalmia of the sheep, ox, goat, and pig and tends to show that the presence of the *Rickettsias* has some etiological significance.

It is not uncommon for a control eye to develop conjunctivitis and contain the parasites, two or three days after the disease has become manifest in the experimentally infected eye. This constitutes more circumstantial evidence that the *Rickettsia* is the cause. Final proof, however, is still lacking.

CONCLUSIONS.

An apparently new form of conjunctivitis of the domestic fowl has been described. No doubt the disease has been seen before and called "ocular roup".

The condition is confined to the structures of the eye. Transmission experiments have led to the successful reproduction of a disease, similar in all respects to that found in nature.

The conjunctivitis is associated invariably with the presence of Rickettsia-like organisms in the conjunctival epithelial cells. Almost identical inclusions have been found in cases of ophthalmia of the sheep, ox, goat, and pig. These fowl parasites have been described and the name, *Rickettsia conjunctivae galli*, proposed for them.

Reasons have been advanced for suggesting that *R. conjunctivae galli* is the true cause of the conjunctivitis.

ACKNOWLEDGMENTS.

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An article dealing with the conjunctival inclusions of pigs is now in the press.

ILLUSTRATIONS.

The smears of conjunctival epithelial scrapings were all stained with Giemsa.

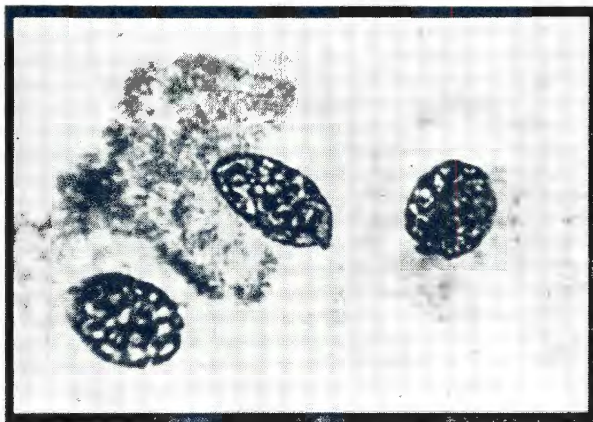


Fig. 1.—1,400 $\times$ . Conjunctival epithelial cell with the left half of its cytoplasm occupied by a colony of *Rickettsia*-like organisms. A clean cell is to the right of the infected cell.

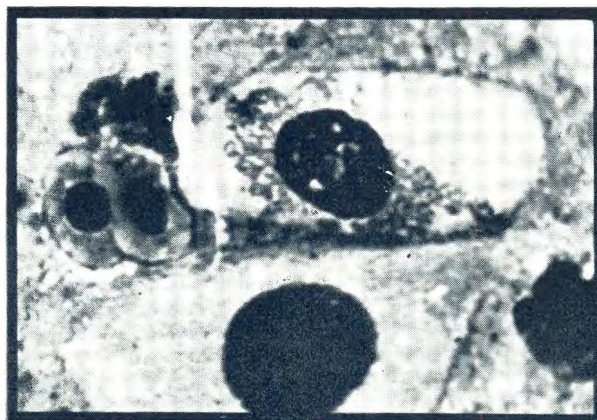


Fig. 2. 1,500 $\times$ . Conjunctival epithelial cell, half full of parasites.



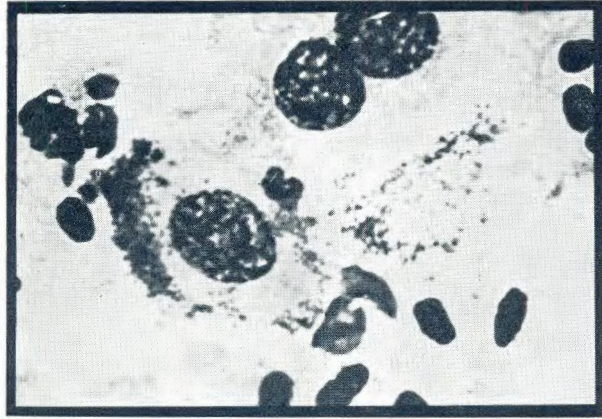


Fig. 3.--1,400 $\times$ . Conjunctival epithelial cell. The organisms are packed closely along the left edge of the cell; this is a common form of grouping. Other parts of the cytoplasm contain a few parasites. To the right of the cell, and outside it, is a scattered mass of Rickettsias.

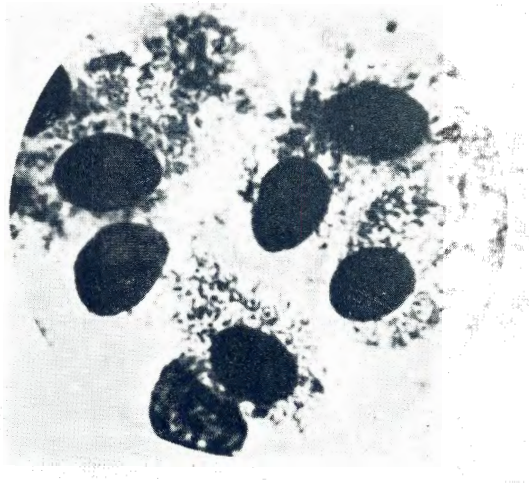


Fig. 4.—1,500 $\times$ . A group of conjunctival epithelial cells containing organisms

CONJUNCTIVITIS IN THE DOMESTIC FOWL.

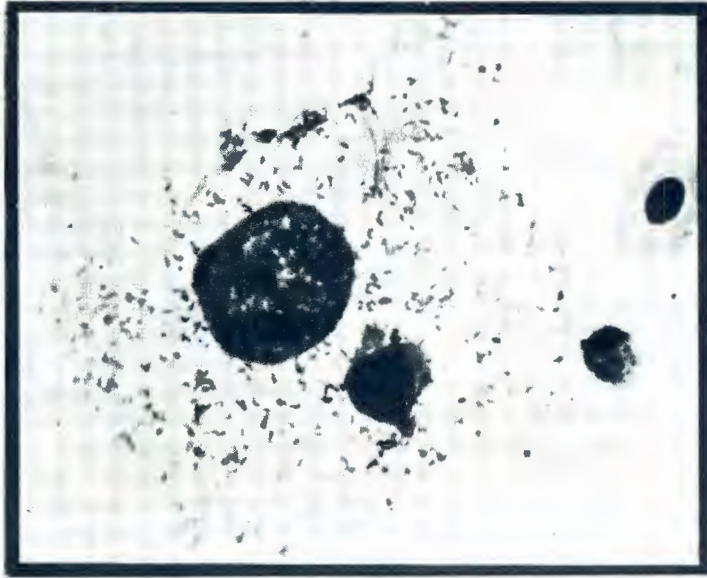


Fig. 5.—1,400 $\times$ . A conjunctival epithelial cell with numerous Rickettsias dispersed evenly throughout the cytoplasm. To the right of, and below the nucleus, is a lump of extraneous nuclear material.

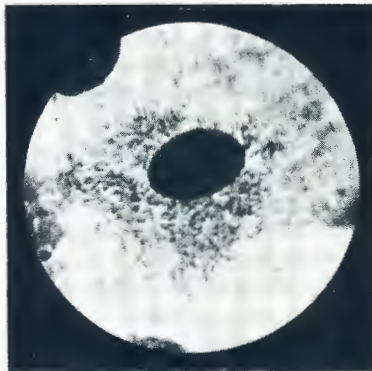


Fig. 6.—1,250 $\times$ . A conjunctival epithelial cell filled with organisms, most of which are rod-shaped.