Isolation of Psittacosis Agents from Follicular Conjunctivitis of Sheep.* (32223)

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Follicular conjunctivitis developed in experimental sheep after parenteral inoculation with a psittacosis-lymphogranuloma-trachoma (PLT) agent responsible for polyarthritis in sheep(1,2). Since many sheep are afflicted with conjunctivitis and further complicating pathologic processes in the eye, attempts were made to isolate PLT agents from sheep with follicular conjunctivitis. These investigations were also undertaken to explore the possible role of conjunctival infection in the epizootiology of PLT infections in animals.

Materials and methods. Four epizootics of follicular conjunctivitis of sheep were investigated in Colorado flocks consisting of 1273, 2400, 1400 and 800 sheep that were 8 to 10 months old. The sheep were examined clinically for other disease manifestations as well as for conjunctivitis.

Collection and cultivation of samples. Conjunctival specimens were collected from 5 or 6 sheep of each herd using trachoma forceps. Samples were taken from both eyes of each sheep, and the forceps with the collected material were washed in 2 ml of sucrose phosphate buffer containing 5,000 μg of streptomycin and 5 μg of vancomycin (Eli Lilly) per ml. An effort was made to collect epithelial cells as well as follicular material by squeezing the conjunctiva with the trachoma forceps. In the laboratory the samples were ground in Ten-Broeck grinders. Three 7-day-old chicken embryos were inoculated *via* the yolk sac route with 0.5 ml amounts of each of the original samples and 10-fold dilutions thereof. The inoculated chicken embryos were incubated and examined as described previously(3).

Cytologic studies. Conjunctival scrapings for cytologic studies were obtained by drawing a dental spatula across the upper tarsal conjunctiva. The scrapings were placed on slides to be stained by the methods of Giemsa or Rice(4), a stain specific for iodine-positive glycogenic material in cytoplasmic inclusions, characteristic for PLT agents of subgroup A and not subgroup B(5). During the initial investigations when our methods of conjunctival sampling were not yet perfected, direct impression smears of the conjunctival surfaces were made at necropsy of experimentally inoculated sheep(2).

PLT infectivity in the blood. Blood samples were collected from each sheep at the time of conjunctival sampling. Parts of the blood samples were treated with sodium ethylenediamine-tetra-acetate (EDTA) for PLT culture in chicken embryos, and serum was collected from the rest.

Identification of isolates from conjunctiva. The effect of the isolated infectious agents on chicken embryos and the tinctorial properties of elementary bodies in yolk sac smears stained by the method of Giménez(6) were used to identify PLT isolates. The strain FC-78 was further characterized in the complement fixation test. Its specific antigenic relationship with PLT strains representative of enzootic abortion of ewes (B-577) and PLT polyarthritis (LW-646) was determined by methods described previously(7).

Results. Follicular conjunctivitis was seen in 90% of the sheep in each of the 4 herds examined. The severity of the conjunctivitis varied and was graded 1+ to 3+. Severity of the first degree (1+) comprised hyperemia of the ocular and palpebral conjunctiva, a small number of prominent follicles on the conjuctiva of the lower and 3rd eyelid in conjunction with moderate serous eye discharge. The 3rd degree of severity (3+) consisted of marked hyperemia in the entire conjunctiva, numerous, often confluent follicles on the lower and 3rd eyelid, the bulbar side of which being padded with follicles. Pannus formation, keratitis and chemosis

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TABLE I. Etiologic, Cytologic and Serologic Findings on Sheep with Follicular Conjunctivitis.

Sheep No.	Degree of follicular conjunctivitis R	L	PLT agent isolated from Conjunctiva	Blood	CF titer*	Polyarthritic signs
1	3+	3+	—	+	16	yes
2	1+	1+	c	+	16	"
3	2+	3+	+	—	64	"
4	3+	3+	—	—	64	"
5	3+ P	3+	+	c	<16	"
11	2+	2+	+	—	128	yes
12	3+	3+	—	+	32	"
13	1+	1+	+	—	128	"
63	3+	3+	—	—	>256	"
67	3+	3+	—	+	128	"
78	3+	3+ P, K	+	—	256	"
86	3+ P, K	3+ P, K	+	+	128	no
89	3+	3+	+	—	<16	"
91	3+	3+	—	+	>256	yes
93	2+	1+	+	—	>256	"
97	3+	3+	+	—	<16	no
100	3+ P, K	3+	—	—	64	"

R, right eye; L, left eye; 1+ to 3+ range of degree of severity; P, pannus; K, keratitis; —, + = PLT culture negative or positive; c, culture contaminated.
* Reciprocal of highest serum dilution with 4+ complement fixation.

were present at this stage. The heavy and seropurulent eye discharge crusted the periphery of the eyes and even sealed the eyelids occasionally. The sheep with this type of eye lesion were photophobic. The second degree of severity (2+) was intermediate between the 2 described stages. The lesions were usually bilateral but sometimes differed in the same animal in severity (Table I). Ten to 25% of the lambs in each of the 4 herds studied had polyarthritis. Almost all lambs with signs of polyarthritis had follicular conjunctivitis.

Conjuctival scrapings of 17 lambs from 3 flocks were cultured. Agents of the PLT group were isolated from 9 (Table I). A definite indication of PLT infection was obtained in 6 of these cases in the 1st yolk sac passage. Three remaining samples were proven to be PLT-positive on subculture. From the whole blood of 6 of the 17 sheep tested, PLT agents were isolated. Only in the case of sheep no. 86 were we able to isolate PLT agents from conjunctival scrapings and blood. PLT infectivity of the conjunctiva and the blood did not correlate in the other instances. The conjunctiva was sampled from more sheep with signs of polyarthritis than from animals with follicular conjunctivitis alone. PLT infectivity in the blood was not detected in the latter group of animals. All of the sheep with PLT infectivity in the blood had significant group-specific complement fixing titers that were 1:128 or higher in 3 instances (Table I). Strain FC-78 isolated from the conjunctiva had a specific antigenic relationship with PLT strains isolated from affected joints of polyarthritis afflicted sheep (8) but not with PLT strains isolated from cases of enzootic abortion of ewes (Table II). Mycoplasma organisms were not isolated from the yolk of chicken embryos that died after inoculation with conjunctival or blood samples.

Inclusions typical of PLT infection were seen in Giemsa stained smears of conjunctival scrapings of 4 sheep (Table I). The elementary bodies had a loose arrangement in the

TABLE II. Comparison of PLT Strains Isolated from Follicular Conjunctivitis, Polyarthritis and Enzootic Abortion of Ewes.

Representative PLT strains	Log_{10} $CELD_{50}$ neutralizing index of hyperimmune serum B-577	LW-646
FC-78 (follicular conjunctivitis)	0	3.0
PA-T-1902 (polyarthritis)	0	3.25
LW-646 (polyarthritis)	1.0	2.5
B-577 (enzootic abortion)	3.0	0

$CELD_{50}$ chicken embryo lethal $doses_{50}$.

inclusions. Structures resembling free elementary bodies were seen frequently in the backgrounds of Giemsa stained conjuctival smears. Iodine-positive inclusions in conjunctival cells were not detected in smears from the 17 sheep listed in Table I.

Discussion. Experimental studies, designed to reveal the effect of PLT agents isolated from joints of polyarthritic lambs on the synovial and other joint tissues, directed our attention to specific conjunctival reactions(1,2). The experimental lambs were 5 to 6 months old, and originated from a closed flock in which follicular conjunctivitis and polyarthritis had not been observed. The inoculum was given either intra-articularly, intravenously or intramuscularly. In addition to polyarthritis, all inoculated lambs developed follicular conjunctivitis. Conjunctival changes were detectable after 3 days, and the highest severity was seen 7 to 14 days after inoculation. Suspensions of the entire conjunctiva yielded PLT agents from 2 of 3 lambs that were examined on the 7th day of the experiment but no PLT agents were isolated from the conjunctiva of lambs subjected to necropsy 3 and 21 days after exposure. A few cytoplasmic PLT inclusions were detected in conjunctival cells from the experimental sheep. The inoculated lambs had a rise of PLT group-specific CF antibodies from less than 1:8 to titers of 1:256 during 7 to 21 days after inoculation(2).

These observations induced us to explore the possibility of conjunctival PLT infections in sheep under natural conditions. Four flocks afflicted with an eye disorder were studied. At least 90% of the sheep involved had follicular conjunctivitis of differing severity. Chemosis, keratitis and pannus formation were associated with the most severe degree of follicular conjunctivitis. PLT agents were isolated from conjunctival scrapings of 9 sheep from 17 animals representing 3 flocks tested culturally.

Signs of polyarthritis were seen in 10 to 25% of the sheep in each of the 4 flocks examined. Polyarthritic lambs always had follicular conjunctivitis; however, in these flocks follicular conjunctivitis was equally severe in the remaining 65 to 80% of the sheep that did not have signs of polyarthritic joint involvement. PLT isolations were achieved from the conjunctiva of animals with and without polyarthritic symptoms. Cultivation of the PLT agent was accomplished more readily from conjunctival scrapings than from suspensions of parts of the entire conjunctiva.

Since the clinical features of the polyarthritis seen in the 4 herds were identical to the one described(8,9) and since PLT agents were isolated from blood of the polyarthritic lambs, it is justified to attribute the polyarthritis in sheep with follicular conjunctivitis to the action of PLT agents. Demonstration of PLT infectivity in the blood with high titers of group-specific CF antibody in the serum was to us an unexpected and interesting finding worthy of further study. In studies on clinically normal sheep, PLT antibodies were not detected if the visceral organs were free of PLT agents even though inapparent intestinal PLT infection may exist in such animals(10).

Our observations also point to the interesting possibility that a common eye infection of sheep may under certain circumstances lead to polyarthritis. The PLT agent isolated from the conjunctiva is specifically related to other PLT strains isolated from joints of polyarthritic sheep. These strains do not have any specific antigenic relationship with PLT agents isolated from enzootic abortion of ewes. Conjunctival PLT infection furnishes an additional explanation for previous findings of distinctly different PLT strains in enzootically infected flocks resulting intermittently in abortions or polyarthritis(11).

As revealed by the investigations presented, PLT agents isolated previously from polyarthritis have a marked effect on the conjunctiva and can be isolated from sheep that have only follicular conjunctivitis and no other clinical symptoms of disease. It is, therefore, important to consider eye infections with PLT agents in the epizootiology of the PLT infections of sheep and possibly other animal species. Murray(12) demonstrated such infections in guinea pigs. Yerasimides (13) and Cello(14) isolated PLT agents from the conjunctiva of cats afflicted with conjunctivitis and pneumonitis. At present it is

not possible to say what relationship may exist between the follicular conjunctivitis which we observed and other ovine eye lesions that were attributed to unidentified rickettsial agents(15). It should not be surprising if PLT agents will be isolated from the latter conditions as was suggested by Dickinson and Cooper(16). However, we cannot see any possibility that the unidentified infectious agent incriminated by Livingston *et al*(17) as causing keratoconjunctivitis in sheep, could be a PLT or rickettsial organism.

Summary. Psittacosis - lymphogranuloma-trachoma agents were isolated from conjunctival scrapings from 9 of 17 sheep representing 3 different flocks afflicted to 90% with follicular conjunctivitis. The PLT agent isolated from follicular conjuctivitis was specifically related to those causing polyarthritis. Ten to 25% of the sheep with eye lesions had polyarthritic signs and the PLT agents were isolated from the blood of 6 of the 17 sheep tested. Parenteral inoculation resulted in follicular conjunctivitis and polyarthritis.

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Comparative Activties of Aspartate Transcarbamylase in Various Tissues of the Rat.* (32224)

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In recent years considerable attention has been given the enzyme, aspartate transcarbamylase (ATC), which catalyzes the first step unique to the biosynthesis of pyrimidines. This enzyme is found widely distributed throughout the plant and animal kingdom including bacteria(1,2), fungi(3), higher plants(2,4), birds(5), and mammals(5,6,7). Of primary concern have been investigations of the possible interactions between pyrimidine end-products and ATC in the regulation of pyrimidine anabolism(6,8,9,10,11). As yet no common denominator for control of pyrimidine biosynthesis is known for all species. Although ATC has been partially purified from certain mammalian tissues and characterized with regard to kinetics and substrate specificity, limited information is available concerning its tissue distributon. However, some localzation studies of this enzyme have been done in certain plants(4) and to a more limited extent in rat tissues(5).

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