

STUDIES ON THE ETIOLOGY OF SPONTANEOUS CONJUNCTIVAL FOLLICULOSIS OF MONKEYS*

I. TRANSMISSION AND FILTRATION EXPERIMENTS

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The *Macacus rhesus* monkey is ordinarily employed for experimental transmission of human trachoma and for study of the inoculation effects of organisms derived from trachomatous tissues. There occurs, however, among certain stock monkeys, spontaneous conjunctival folliculosis which may simulate the experimental disease induced by the inoculation of suspensions either of human trachomatous tissues or of *Bacterium granulosis* (1, 2). Nevertheless, as has been mentioned previously (1, 3), it is possible to obtain reliable experimental results with trachomatous material by the selection of animals with clear conjunctivae, and their careful quarantine.

Spontaneous conjunctival folliculosis of monkeys was described by Hess and Römer in 1906 (4). They believed that it differed from experimental trachoma, as induced by inoculation of suspensions of human trachomatous tissues, in that it exhibited less numerous, smaller, and more superficial follicles, with less destruction of epithelium. They stated that while spontaneous folliculosis did not resemble human trachoma, either clinically or anatomically, experimental trachoma showed a close similarity to the disease in man.

The spontaneous conjunctivitis was also known to Nicolle and Lumbroso (3, 5) who reported in 1926 that by scarification of the palpebral conjunctiva of an affected eye, the lesions spread and after a short period of time follicles appeared in the conjunctiva of the other eye. The follicular contents, when inoculated into normal conjunctivae of fresh monkeys, induced a similar reaction; yet these investigators believed that there might be a "telluric" origin of the disease.

Wilson (6), in 1928-29, failed to confirm Nicolle and Lumbroso's finding of the spread of follicles by mechanical means and suggested, on the basis of clinical

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experience, that the condition might not be telluric but infectious in origin. He studied fifteen monkeys of different species kept in the Zoological Gardens at Cairo, Egypt, of which twelve manifested folliculosis in either of two forms: Type I showed simple, non-inflammatory, discrete follicles scattered over the palpebral conjunctiva and probably enlarged lymphoid nodules, and Type II "inflammatory" conjunctivitis characterized by viscid secretion and larger follicles which were at times hemorrhagic. In neither case were the follicles present over the tarsal plate. Wilson regards as criteria for the diagnosis of trachoma, the presence of follicles over the tarsal plate; their rupture either spontaneously or on pressure; conjunctival scarring; and corneal pannus. In view of the fact that he found none of these signs in the spontaneous disease, he considered the latter not as trachoma but as folliculosis. Yet he could make no histological distinction between the two in their early stages.

Weiss (7) (1930) and Thygeson (8) (1932) also described spontaneous folliculosis, although Thygeson met with it in only three *Macacus rhesus* monkeys. The lesions were unaccompanied by signs of inflammation, their contents failed to infect normal monkeys, traumatism did not cause their generalization, and finally, the follicles were rapidly absorbed, leaving the conjunctiva normal. The histological structure of the spontaneous disease differed from that of experimental trachoma in being less pronounced in degree and giving rise to less cicatricial tissue and involvement of the tarsus.

Our own observations have shown that folliculosis, especially Type I of Wilson (6), is widespread among certain groups of animals received from dealers, particularly those housed under crowded and unclean conditions.

The foregoing review indicates that a divergence of opinion exists with respect not only to the cause of spontaneous folliculosis but also to its clinical and pathological identity. We undertook its study with the aim of determining, if possible, the incitant. In this first paper we shall describe the clinical and pathological appearance of the spontaneous disease and deal with the problem of whether it is disseminated by an infectious agent, and if so, under what conditions. The results here reported are based on observations upon over 250 *Macacus rhesus* monkeys.

Types of Spontaneous Conjunctival Folliculosis

Confirming the observations of Wilson (6), we have found that spontaneous folliculosis may exist in two distinct forms. Type I, the more widespread, is characterized by the appearance of one or more discrete follicles usually situated retrotarsally, but also at any point in the palpebral conjunctiva except over the tarsus. The follicle of this

type is small, hard, shiny, and superficial. It is often transitory, as a rule lasting from 1 to 5 or 6 weeks. There is no other sign of inflammatory reaction in the surrounding tissues. The lesions may be regarded, in accordance with Wilson, who made histological examinations, as a normal hypertrophy of preexisting lymph nodules of the conjunctiva. We have found, as he did, that mechanical manipulation of the lids does not induce generalization, or spread, of follicles and we have failed, like Thygeson, to transfer the affection to fresh monkeys.

In our earlier studies on experimental trachoma, simple folliculosis (Type I) was considered as without positive significance when found to occur in test animals. Olitsky, Knutti, and Tyler (9) state that "a decision as to the positive or negative character of the results was rendered difficult by the mild follicular type of reaction which some of the animals exhibited. . . . Inasmuch as true experimental trachoma is progressive and persistent, only those reactions which endured for long periods of time could, on the basis of our present knowledge, be considered positive."

It would appear, therefore, that this simple condition, which is readily recognizable and clearly differentiated from the other form of folliculosis and from experimental trachoma, need not detain us. On the other hand, Type II, the lesions of which are less commonly met with in nature and which have distinctly progressive and persistent reactions, is worthy of further study and has provided the subject of the present investigation. Therefore, all reference to "folliculosis" henceforth relates to this Type II.

Clinical Appearance.—The essential characteristics of spontaneous folliculosis of Type II are slow progression with infrequent exacerbations; persistence, lasting usually throughout the life of the animal; and marked inflammatory reaction of the conjunctiva.

In a fully developed case occurring in a stock animal, the disease becomes evident only upon eversion of the lids. The upper and lower conjunctivae of both eyes are usually affected. At the inner canthus there may be a drop or two of faintly yellowish, mucoid secretion. The palpebral conjunctiva, including the area over the tarsal plate which, as a rule, is less involved, is congested and appears brownish red. The vascular structures are obliterated and there is considerable edema with thickening of the lid, especially the upper one. The entire surface is covered by large, greyish yellow, gelatinous follicles which

are often agglomerated into irregular masses. When pressed upon with a glass slide, they rupture and expel a greyish white, grumous material consisting chiefly of cellular elements. Occasionally the granulations are dark red and hemorrhagic. In some animals the lesions over the tarsus are smaller and more regular; in others this area appears only roughened and sandy. In well advanced cases the conjunctiva is thickened, the vessels are injected, large discrete and papilliform confluent follicles are present in the palpebral conjunctiva, and the secretion is either diminished or absent. Neither pannus, nor definite visible scars, ulcers, or pits are seen; but in many instances follicles appear on the bulbar conjunctiva.

Histopathology.—The epithelium is thinned out or denuded over most of the surface; but in some areas large goblet cell formation along with thickened epithelium may be seen. A varying degree, but never a marked one, of subepithelial cellular infiltration is visible, consisting chiefly of lymphocytes together with some clasmatocytes and a few plasma cells. The follicle contains an outer zone of lymphocytes, beyond and among which are scattered plasma cells. The central area appears lighter, owing to the closely packed mass of clasmatocytes—cells with vesicular nuclei, without much chromatin, and with fairly clear, homogeneous cytoplasm. The fresher lesions contain in the central area, besides clasmatocytes, a few primitive connective tissue cells and monocytes. Mitotic figures are often present. There are fine bands of connective tissue surrounding the follicles with slender strands entering the latter. The lesion may be considered as a folliculoma rather than a granuloma, for granulation tissue and giant cells are absent.

Bacteriology.—Bacteriological examination of affected conjunctival tissue yields a variety of microorganisms. These will be described in a succeeding paper. At this point, however, we wish to remark that *Bacterium granulosis* (1, 2) has not been encountered in spontaneous folliculosis, although over 50 animals have been studied for the express purpose of isolating this microorganism.¹

¹ We desire to state here that in addition to several indifferent varieties of bacteria we have isolated and cultivated from spontaneous conjunctival folliculosis of monkeys a microorganism capable of reproducing the disease in normal monkeys. The organism in question is a minute, aerobic, actively motile, encapsulated, Gram-negative bacillus, having a single (sometimes double), polar, long flagellum. The cultural and pathogenic properties of this new species of bacterium will be described in a subsequent paper.

*Transmission Experiments*²

Transmission by Inoculation of Tissue Suspensions.—The conjunctival tissue of monkeys having spontaneous folliculosis was removed, prepared for inoculation, and injected subconjunctivally in the left upper lid of normal *Macacus rhesus* monkeys after the manner of Noguchi (2). The results were uniform in practically all cases.

Within 24 hours after inoculation, marked acute conjunctivitis developed. This was accompanied by pronounced edema and considerable thickening of the lid which extended so as to include 2 or 3 cm. of the cutaneous tissue over the outer canthus and the supraorbital ridge. Abscess formation rarely occurred. After 5 to 7 days the acute process subsided but the eyelid still drooped and the skin pitted on pressure. On eversion, a definite greyish yellow discharge was seen. The upper conjunctiva was markedly roughened, edematous, hyperemic, and purplish red in color. At this early stage large and small follicles were visible, the larger granulations appearing retrotarsally and the smaller ones over the tarsus, and the tortuous course of the preexisting blood vessels could be perceived. The remaining uninoculated conjunctivae exhibited congestion, slight edema, and roughened surfaces. After another week the upper and the two lower uninoculated conjunctivae showed the follicular reaction, while the inoculated lid became more markedly involved. From 14 to 21 days later, all the conjunctival tissue exhibited the clinical and anatomical changes characteristic of the fully developed spontaneous folliculosis observed in stock animals.

The following protocol summarizes the results of the tests.

In the first instance individual suspensions of the conjunctival tissues of seven stock monkeys having folliculosis were inoculated into twenty-four normal animals, two to four serving for each test. The tissue of the seven stock monkeys induced folliculosis in twenty-two of the twenty-four monkeys employed. The material of an eighth affected animal was without effect in either of two monkeys. Thus, including this last mentioned exceptional result, of twenty-six monkeys inoculated with material from eight affected stock animals, twenty-two developed characteristic folliculosis.

The second test consisted of inoculations into normal monkeys of tissues derived from the experimentally induced lesions. By this means the follicular conjunc-

² All operative procedures were carried on with the aid of ether anesthesia.

tivitis was propagated through eight passages to date. The individual suspensions of tissues from thirteen monkeys with experimental folliculosis were injected into twenty-four animals—two for each case except in two instances, in which only one animal was inoculated—and all twenty-four showed marked, characteristic conjunctival folliculosis.

Transmission by Swabbing Secretions on the Conjunctiva.—

In still another experiment, a cotton swab was applied to the lesions of an affected stock monkey and the secretion thus secured transferred directly, by gentle rubbing, to the smooth upper left conjunctivae of two monkeys. After six such swabbings, over a period of 6 days, folliculosis was manifest in both animals: the treated eye revealed first signs of the affection on the 3rd day after the last swabbing; the untreated, on the 10th day.

In summarizing these results, we find that only one of twenty-two animals having folliculosis yielded material not capable of producing the disease, and that of 52 monkeys inoculated with suspensions, or swabbed with the secretions, of affected conjunctival tissues, forty-eight showed signs of folliculosis indistinguishable from those seen in nature. Hence it appears that the *Macacus rhesus* monkey is highly sensitive to the active agent of the disease. The inference is that folliculosis is infectious, a view supported by the results of the contact experiments to be described.

Control Experiments.—Olitsky, Knutti, and Tyler (1, 9–12), Finnoff and Thygeson (13), and others have reported numerous control tests on monkeys in which different materials, such as human and monkey conjunctival tissue, culture media, saline solution, and various species of bacteria, have been injected subconjunctivally with uniformly negative results. We have observed that mere scarification of smooth, normal conjunctivae has not been followed by folliculosis.

During the course of our studies we inoculated twenty-one monkeys with bacteria encountered irregularly in cultures derived from folliculosis tissues, consisting chiefly of Gram-negative, chromogenic bacilli, but without specific effect; and we injected seven monkeys with microorganisms obtained from human non-trachomatous conjunctivitis, likewise with non-specific results. Cultures of three organisms secured from cases of trachoma in Tunis and sent us by Dr. Thygeson were also without effect in nine monkeys.

Suspensions of normal monkey conjunctival tissue were inactive in ten animals and Berkefeld V filtrates of similar materials were non-pathogenic in four.

Four monkeys injected with saline solution washings of sterile agar slants, and

two swabbed conjunctivally for 5 consecutive days with material from cages in which they were confined failed to show folliculosis.

From the results obtained in the 57 monkeys employed in this series of tests, it would appear that the disease is not set up in normal conjunctivae by the injection of various control materials. The effects of inoculating tissue suspensions derived from cases of spontaneous folliculosis would appear to be specific.

Contact Experiments.—In view of the fact that selected monkeys with smooth conjunctivae kept in quarantine did not show folliculosis, and did not develop the disease after injection of non-folliculosis materials, the following positive results of contact experiments take on significance.

Three experiments were made. In each, two monkeys having smooth conjunctivae were caged with two animals showing folliculosis. 2 to 10 weeks later the six test animals that had been previously normal exhibited characteristic follicular conjunctivitis in both eyes.

We have already commented on the fact that the disease can be spread by contact from the inoculated conjunctiva to the uninoculated membranes of the same monkey. From the foregoing experiments it is clear that spontaneous folliculosis can be conveyed from affected to normal animals by simply caging the two together.

The fact that the disease can be transmitted to normal animals by contact brings out the difficulties of maintaining quarantine, especially when monkeys are handled during experimentation. That we succeeded in overcoming these difficulties is demonstrated by the small number of incidental follicular infections that developed, namely, four in a group of 250 animals. These were observed during the early stages of the investigations after the animals had been released from quarantine.

Filtration Experiments

We next attempted to determine whether the active agent of spontaneous folliculosis will pass through filters that retain ordinary bacteria.

Ten separate filtration tests were made with Berkefeld V filters and other more permeable filters of the same sort, designated in Table I as "VV," which permitted

the passage of *B. prodigiosus* (14). In further tests Seitz single disc filters were also used. The material for filtration consisted of fragments of excised conjunctival tissue, removed from monkeys having fully developed spontaneous folliculosis, suspended by grinding in either physiological saline solution or hormone broth. As a rule, fragments of the conjunctivae of four lids were ground in 5 to 10 cc. of the suspension fluid and, after sedimentation of the larger particles of tissue, the faintly clouded supernatant fluid was transferred to the filtering apparatus. Filtration was made at a negative pressure of 60 cm. Hg and continued until the entire suspension had passed into the filter candle or disc—the

TABLE I
Results of Filtration Tests

No. of experiment	Type of filter used	Fluid used for suspension	Results of inoculation of monkeys			
			With unfiltered material		With filtered material	
			No. inoculated	No. showing folliculosis	No. inoculated	No. showing folliculosis
1	Berkefeld V	Saline solution	2	2	3	0
2	"	" "	2	2	3	0
3	{ " V	" "	2	2	{ 2	0
4	{ " VV					
5	{ " V					
6	{ " VV					
7	{ " V					
8	"	Hormone broth	2	2	3	0
9	Seitz	{ " "	1	1	{ 2	0
10	"	{ Saline solution				
		{ Hormone broth				
		{ Saline solution	1	1	{ 2	0

procedure requiring usually less than 10 minutes. We hoped that by these means the infectious agent passing into the filtrate might be not too greatly diluted for pathogenicity. The results of these tests are summarized in Table I.

A summary (Table I) of the results of filtration experiments on 50 monkeys indicates that all of eighteen animals inoculated with unfiltered folliculosis material developed characteristic folliculosis. On the other hand, none of thirty-two monkeys injected with filtrates obtained in various ways, including those secured from very permeable filters, showed the disease. The active agent failed to pass even through filters that let through *B. prodigiosus*.

Absence of Inclusion Bodies.—Many filtrable viruses have distinctive cytotropic effects (15) and cellular inclusion bodies are characteristically associated with the action of some of them. As the following protocol shows, no such bodies have been found in the cells derived from spontaneous folliculosis cases.

Film preparations were made from fourteen monkeys showing early folliculosis. Lesions within 4 weeks after their onset were examined because the so called inclusion bodies of granular conjunctivitis of various types are found most often in the first stages of the disease. Films stained by the method of Giemsa, from one to three slides in each case, were examined with the aid of a mechanical stage, the entire film being searched. No inclusion bodies were found,³ nor were such bodies discoverable in any of the large number of histopathological sections of affected conjunctival tissues, stained by Giemsa's method and by eosin-methylene blue.

CONCLUSIONS

Spontaneous folliculosis of *Macacus rhesus* monkeys—a type of follicular conjunctivitis associated with marked, local, inflammatory reactions—is apparently a disease *sui generis*, due to a specific infectious agent. It can be transmitted from monkey to monkey by means of subconjunctival injection of suspensions, and by conjunctival swabbing of the secretions, of affected tissues, or by contact of normal animals with folliculosis monkeys.

The agent causing folliculosis has failed in our hands to pass through Berkefeld and Seitz filters, even those of an unusual degree of permeability; and the lesions that it causes show no cellular inclusions suggestive of the action of a virus. The condition is due apparently to an organism of low grade pathogenicity. The essential histopathological structure corresponds to that of a folliculoma (16) which, while not identical with a granuloma, bears certain resemblances to the latter.

The studies here reported concern only one species of monkey, *Macacus rhesus*. Further investigations will be carried out on different species of Anthropeoidea and other animals.

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³ We are indebted to Dr. R. E. Knutti for his examination of the slides from five of the monkeys.

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