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Tuberculosis in a Rhinoceros

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The report on the case of tuberculosis in a rhinoceros is very few and only one case caused by the bovine type of tubercle bacillus has been reported by Griffith (1939).[1] Recently, the authors have encountered the first case of tuberculosis in a rhinoceros in our country and obtained the human type of tubercle bacillus. Streptococci were also isolated, almost in a pure state, from the contents of the frontal sinuses and nasal conchae of the animal. This report deals with the bacteriological examinations carried out with both the acid-fast bacillus and the streptococcus isolated from the animal.

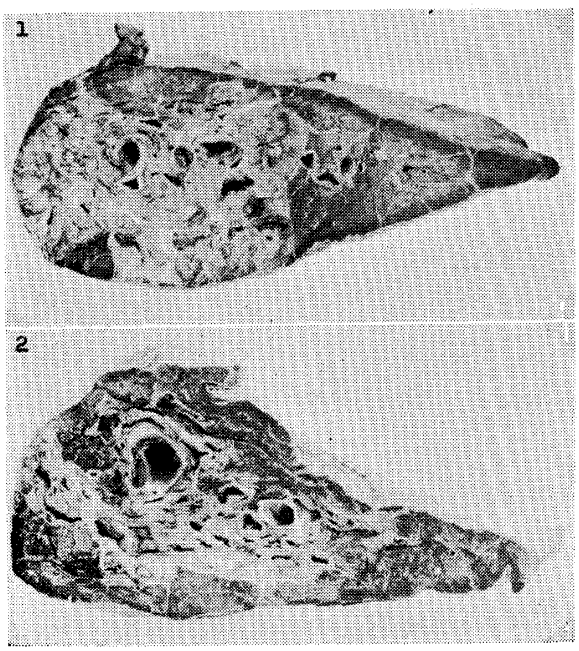
Brief history of the rhinoceros and its clinical and pathological findings.

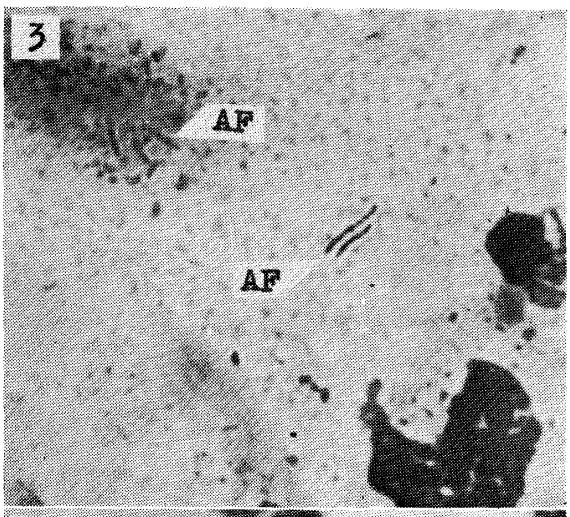
The animal (male, age unknown) was brought over from Africa to Japan in 1955 and had been kept in a zoological garden in the city of Osaka till it died. In January, 1961, the animal began to show tachypnea and became to take much time in mastication. From June, it began to excrete mucous nasal discharge or sputum every morning and this status lasted almost four months. Microscopic examination of the nasal discharge always revealed the presence of a large number of acid-fast bacilli and isolation of the bacilli from the specimen both by cultivation and by guinea pig inoculation was always successful. The animal died on October 20th, 1961. Immediately after its death, the carcass was forwarded to our department and pathological and bacteriological examinations were carried out. At autopsy, many abscesses, varying in size from soybean to walnut, were found in every lobus of the lungs (Figs. 1 and 2). Most of the abscesses were occupied by creamy contents. Cavity formation was observed in some abscesses. Catarrhal changes were conspicuous in pulmonic parenchyma. Bronchial lymph nodes were somewhat edematous. Remarkable calcareous deposition was observed in endocardium, aortic and pulmonic semilunar valves, and in bicuspid and tricuspid valves. Atrophy was marked in fatty tissues, especially in those of heart, in liver and spleen. Hemosiderosis was also remarkable. Catarrhal changes were also observed in frontal sinuses and nasal conchae, all of which were occupied by pus. Microscopic examination revealed the presence of a large number of acid-fast bacilli in lesions of lungs. Numerous streptococci, together with acid-fast bacilli, were found in pus taken from frontal sinuses and nasal conchae.

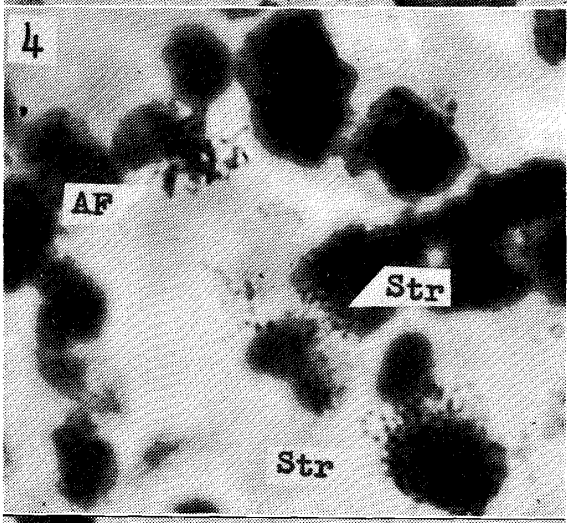
Isolation of the organisms.

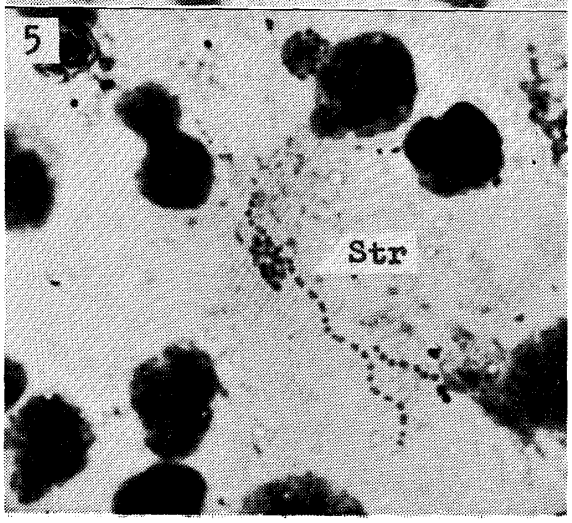
The methods used for the isolation of the acid-fast bacillus were the same as those described elsewhere,[2,3] that is, direct cultivation, cultivation after treatment of the sample with a 5% sulfuric acid solution or with a 4% sodium hydroxide solution, and guinea pig inoculation. For the purpose of isolating the tubercle bacillus and in order to examine for the

* Deceased on July 16, 1963.









Figs. 1 and 2. Tuberculous lesions in cut section of lungs.

Fig. 3. Acid-fast bacilli (AF) in smear of lesion in lung.

Figs. 4 and 5. Acid-fast bacilli (AF) and streptococci (Str) in smear of pus taken at autopsy from nasal concha.

effect of glycerol on primary growth of the bacillus, two types of Loewenstein-Jensen medium, containing glycerol and no glycerol, were prepared. On primary isolation, the growth of the bacillus on medium containing no glycerol was relatively good, but on medium containing glycerol more abundant.

The streptococcus was isolated on agar plate from the sample taken out of the frontal sinus.

I. The acid-fast bacillus.

The strain used for the following bacteriological examinations was one of those isolated on Loewenstein-Jensen medium containing no glycerol.

a. Cultural characters. As shown in Table 1, the bacillus grew as well on Petragnani medium as on Loewenstein-Jensen medium. The addition of glycerol to both egg media improved its growth. The growth, however, was not rapid, but rather slow and usually 10

Table 1. Growth of the isolated acid-fast bacillus (4 weeks at 37°C)

Strain / Culture medium	Isolated	Control								
		Human type			Bovine type		Avian type	Saprophyte		
		H 37 R	Aoyama B	MK -1h	RO	Bov	AF	No. 22	No. 39	No. 46
Loewenstein-Jensen	++	++	++	++	++	++	+++	+++	+++	+++
" (without glycerol)	+	++	+++	++	++	++	++	+++	+++	+++
Petragnani	++	++	++	++	++	++	+++	+++	+++	+++
" (without glycerol)	+	+	+	++	++	++	+++	+++	+++	+++
Dubos	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Kirchner	++	++	++	++	++	++	+++	+++	+++	+++
Sauton	+	+	±	+++	+++	++	+++	+++	+++	+++
Coagulated ox serum	—	—	—	—	++	±	+++	+++	+++	++
Glycerol potato	—	—	+	+	++	++	+++	+++	+++	++
Glycerol agar	++	++	++	++	+	++	+++	+++	+++	++
Nutrient agar	—	±	±	—	—	—	+++	+++	+++	++
Glycerol broth	±	+	±	+	+	±	+++	+++	++	++
Nutrient broth	—	+	+	+	+	±	+	+	+	+
Peptone water	—	±	±	±	—	—	+	+	+	+

to 14 days elapsed before growth was apparent. The appearance of growth on egg media after 4 weeks' incubation at 37°C was quite similar to that of the control human type. The bacillus also grew well in Dubos medium and on the surfaces of Kirchner, Sauton and glycerol agar media, but scanty or no growth on coagulated ox serum, glycerol potato, nutrient agar, nutrient broth and peptone water. The control bacilli of the avian type and saprophyte generally grew rapidly and abundantly on these media.

b. Biochemical characters. As shown in Table 2 ,the isolated bacillus gave a high *Kochfestigkeit* (Kf) value[4] and a positive neutral red reaction[5]. Catalase production was positive, but weak. Niacin production tested by the method of Konno[6,7] was positive. The resistance of the bacillus to streptomycin, *p*-aminosalicylic acid, and isoniazid was similar to those of the control human and bovine type bacilli. The growth on Sauton agar containing potassium tellurite[8] was completely inhibited.

c. Animal inoculation. The pathogenic activity of the isolated bacillus to laboratory

Table 2. Biochemical properties of the isolated acid-fast bacillus

	Strain	*Kf**1 (min)	Neutral red	Catalase	Niacin	Resistance to: SM*2 (γ/ml)	PAS*3 (γ/ml)	INH*4 (γ/ml)	Potassium tellurite*5 0.025%	0.05%
	Isolated	15	+	+	+	10	<10	<0.1	—	—
Control	H 37 R	14	+	+	+	5	10	<0.1	—	—
	Aoyama B	10	+	+	+	10	<10	0.1	—	—
	Mk-1h	10	+	+	+	5	10	<0.1	—	—
	RO	14	+	+	—	10	<10	<0.1	—	—
	Bov	5	+	+	—	10	10	<0.1	+	—
	AF	5	—	++	—	1	·	·	++	+
	No. 22	1	—	++	—	20<	·	·	+	+
	No. 39	1	—	++	—	20<	·	·	++	++
	No. 46	1	—	++	—	20	·	·	++	++

*1···*Kochfestigkeit*
*2···Dihydrostreptomycin sulfate (Dubos medium)
*3···*p*-Aminosalicylic acid (Loewenstein-Jensen medium)
*4···Isoniazid (Dubos medium)
*5···Sauton agar .

Table 3. Pathogenicity of the isolated acid-fast bacillus for laboratory animals

Animal	Body wt. (Kg) and sex	Organism injected (mg)	Tuberculin reaction	Sacrificed (Days after injection)	Tuberculous finding at autopsy: Site of injection	Lung	Liver	Spleen	Kidney	Lymph nodes: Inguinal	Mesenteric	Axillary	Cervical	Lumbar	Bronchial	Hepatic
Guinea pig	0.30 ♂	5	+	30	++	++	++	++	—	++	+	+	—	+	++	+
	0.50 ♂	2	+	29	++	—	+	++	—	++	±	±	—	+	+	+
	0.45 ♂	2	+	32	++	+	++	++	—	++	+	±	+	+	+	+
Rabbit	1.30 ♂	5	+	33	+	—	—	—	—	+	—	—	—	—	—	—
	1.75 ♂	2	+	29	+	—	—	—	—	+	—	—	—	—	—	—
	1.50 ♂	2	+	47	+	—	—	—	—	—	—	—	—	—	—	—
Fowl	0.90 ♂	5	·	32	—	—	—	—	—							
	1.20 ♂	2	·	32	—	—	—	—	—							
	1.10 ♂	2	·	32	—	—	—	—	—							

animals was examined. The results are shown in Table 3. The bacillus was remarkably pathogenic to guinea pigs, but not so to rabbits in which only local lesions were formed. To fowls the bacillus was nonpathogenic.

From the results of the cultural, biochemical, and animal inoculation experiments, it is concluded that the acid-fast bacillus isolated from the rhinoceros belongs to the human type of tubercle bacillus.

II. The streptococcus.

The streptococcus was isolated only from pus contained in nasal conchae and frontal sinuses and not from other organs. Though it was apparent that the principal cause of death of the animal was due to infection of the human type tubercle bacillus, it was thought important to examine for the properties of the streptococcus isolated from specimen taken at autopsy and to make clear whether the streptococcus participated in death of the animal. For that account, bacteriological experiments were carried out with the streptococcus. Unless otherwise specified, the basal medium used for the experiments was the meat infusion broth, pH 7.6, and incubation temperature was 37°C.

a. Cultural characters. The streptococcus grew on meat infusion agar, forming a minute colony after 24 hours' incubation. After 48 hours the colony extended in diameter. The addition of defibrinated blood to the medium improved its growth. The growth in broth was good, forming a granular sediment. In glucose broth the growth was more abundant.

b. Biochemical characters. As shown in Table 4a, the isolated streptococcus exhibited β-hemolysis on blood agar plates. The hemolytic activity was not influenced by the addition of glucose to the medium. The streptococcus was not resistant to heat and did not grow in broth containing bile or in broth of high pH. Growth was recognized neither in broth containing 6.5% sodium chloride nor in milk containing 0.1% methylene blue. Bacitracin (1 u/ml) did not inhibit its growth, but penicillin (0.1 u/ml) completely did.

Table 4a. Biochemical properties of the isolated streptococcus

Strain		Hemolysis				Resistance to :							
		Horse and rabbit red cells		Goat red cells		Heat (30 min)		40% Bile	pH 9.6	6.5% NaCl	0.1% Methylene blue	Bacitracin (1 u/ml)	Penicillin (0.1 u/ml)
		Glucose		Glucose		55°C	60°C						
		−	+	−	+								
Isolated		β	β	β	β	−	−	−	−	−	−	+	−
Control	*Str. equi* Kitasato	β	β	β	β	−	−	−	−	−	−	−	−
	Str. pyogenes 089	β	β	β	β	−	−	−	−	−	−	−	−
	Str. pyogenes Richard	·	·	·	·	−	−	−	−	−	−	−	−
	Str. pyogenes Group B	·	·	·	·	−	−	−	−	−	−	−	−

Table 4b. Biochemical properties of the isolated streptococcus

Strain		Decomposition												Final pH in glucose broth
		Trehalose	Sorbitol	Mannitol	Lactose	Sucrose	Rhamnose	Salicin	Inulin	Starch	Gelatin	Esculin	Sodium hippurate	
Isolated		+	−	−	−	+	−	−	−	+	−	±	−	4.4–4.8
Control	*Str. equi* Kitasato	−	−	−	−	+	−	+	−	±	−	±	−	4.4–4.8
	Str. pyogenes 089	±	±	±	−	±	−	+	−	+	−	+	−	4.4–4.8
	Str. pyogenes Richard	±	−	−	−	±	−	±	−	−	−	+	−	4.4–4.8
	Str. pyogenes Group B	+	−	−	−	+	−	−	−	−	−	+	−	4.4–4.8

Though not shown in the table, the isolated streptococcus, as well as the control streptococci, gave a negative catalase reaction. As shown in Table 4b, when tested in Hiss's serum water containing 0.5% sugar, the isolated streptococcus produced acids from trehalose, sucrose and starch. Gelatin and sodium hippurate were not decomposed. Split of esculin was not distinct. The final pH in glucose broth was similar to those of the control organisms.

c. Animal inoculation. A saline suspension of pus taken at autopsy, containing streptococci, was prepared. Injection of the suspension into mice did not cause in these animals any infection due to the cocci. After isolating a pure culture of streptococcus, an inoculation test in mice was carried out, again, As a result of the test it was proved that the isolated streptococcus was nonpathogenic to mice.

From these results, it is assumed that the streptococcus isolated from the rhinoceros is one of the commensals belonging to type III of the streptococci classified by Ochi et al.[9,10] and that, though participated to some extent in death of the animal, it was not the principal causative agent.

Summary

A rhinoceros which had been kept in a zoological garden in the city of Osaka died from tuberculosis. From the nasal discharge taken during life and from various specimens taken at autopsy, acid-fast bacilli were isolated. Bacteriological examination proved that the bacilli belong to the human type of tubercle bacillus.

Hemolytic streptococci were also isolated from specimens taken at autopsy from the animal. The cocci, however, seemed to be one of the commensals and not directly concerned with death of the animal.

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