



## 猿の一結核例について

メタデータ	言語: English 出版者: 公開日: 2009-08-25 キーワード (Ja): キーワード (En): 作成者: 高木, 静雄, 尾藤, 行雄, 岡, 武哲, 広直, 武司 メールアドレス: 所属:
URL	<a href="https://doi.org/10.24729/00009643">https://doi.org/10.24729/00009643</a>

## Tuberculosis in a Monkey

By

Shizuo TAKAGI, Yukio BITO, Takenori OKA and Takeshi HIRONAO

Department of Veterinary Science, College of Agriculture

(Received September 30, 1955)

On the cases of spontaneous tuberculosis in monkeys, many authors (Rabinowitsch 1906, Griffith 1929, and Schroeder 1938, etc.) have reported and reviewed in detail and we can refer to them.

In Japan, reports on such cases are encountered but rarely and we can find merely those reported by Oka (1926) and Yamaguchi and his co-workers (1954).

Recently (1952), having a chance to autopsy a carcass of a spontaneously dead monkey in whose various organs tuberculous lesions were observed, and obtaining from the tuberculous material a pure culture of acid-fast organism suspicious to be causative, the authors carried out more precise pathological and bacteriological examinations with those materials obtained, and attained to a certain result, which will be presented below.

### **The history of the monkey and its clinical findings**

The monkey examined was a Kanikui-zaru (*Macaca irus*; female, age unknown). It was brought over in summer, 1951, from Malaya to Japan, thenceforth it was kept as a pet by a merchant family in Sakai. On July 12, 1952, as the monkey suddenly showed coughing, depression and anorexia, it received clinical inspection of a veterinarian. At that time, the temperature was 38°C, the pulse weak, the respiration dyspneic and auscultation revealed crepitate *râle* in the bronchial tract. Palpation revealed also the presence of a hard nodule in the abdomen. The monkey was, then, further forwarded to the veterinarians of the Tennoji Zoological Gardens, Osaka, and there it received clinical inspection again, and was suspected of catarrhal pneumonia. On July 15, the monkey died and the following day, the carcass was forwarded to us to make pathological and bacteriological examinations.

### **Autopsy and histopathological findings of the monkey**

The monkey was 1.7 kg in weight, 24 cm in length, and was in the state of malnutrition and lean. Its conjunctiva and natural openings were cyanotic. No other remarkable external findings were obtained.

The distribution of lesions observed at autopsy is shown in Table 1.

Table 1. Autopsy and histopathological findings of the monkey.

Findings Organs	Autopsy		Histopathological findings					
	Miliary tubercles	Large tubercles	Caseation	Calcification	Epithelioid cells	Lymphocytes	Encapsulation	Acid-fast bacilli
Lung	++	—	+	—	±	+	+	+
Liver	++	—	++	—	±	+	++	+
Spleen	+++	—	++	—	±	+	++	+
Kidney	+	—	+	—	±	+	+	—
Stomach, Duodenum, Jejunum	—	—	—	—	—	—	—	—
Ileum	++	—	—	—	+	+	±	++
Caecum, Colon, Rectum	—	—	—	—	—	—	—	—
Bronchial l.n.	+	—	+	—	±	+	+	+
Portal l.n.	+	—	+	—	±	+	+	+
Splenic l.n.	+	+	+	—	±	+	+	+
Mesenteric l.n.	—	+++	+++	+	±	+	+++	+

l.n.……Lymph nodes.

In lungs, the mixed form of exudative and productive inflammations was observed. Many miliary tubercles were with caseous centers surrounded by a small number of Langhans' giant cells and epithelioid cells which contained acid-fast bacilli in small numbers. Lymphatic exudation was observed but slight and the fibroplastic zone was considerably thin. By the silver staining technique, dense reticulum fibers were observed. Softening of cavities was not observed. These tubercles, some of which conglomerated, are considered to be the exudative lesions in alveoli containing a number of monocytes, and marked fibrinous exudates. Enlarged bronchial lymph nodes contained miliary tubercles.

Adhesion of the left lobe of the liver to the omentum was observed. Many typical miliary tubercles, some conglomerated, were observed to have been scattered throughout the whole liver, consisting of necrotic centers, epithelioid cells and giant cells containing acid-fast bacilli and consisting of encapsulation with lymphatic fibrinoplastic zones. Some of the tubercles were those which consisted of epithelial cells and a number of giant cells. Increase of reticulum fibers, which had not yet been collagenized, was observed especially in the outermost layer of tubercles.

Portal lymph nodes were hard and enlarged, containing small areas of caseation, many epithelioid cells and a number of giant cells.

In the spleen, numerous tubercles, solitary and conglomerate, were observed. The splenic parenchyma was in the state of atrophy, containing numerous erythrocytes. The findings in the splenic lymph nodes were similar to those which were observed in the portal lymph nodes.

The enlarged mesenteric lymph node, 2.5 by 3.5 by 2.1 cm in size, was observed to have consisted of numerous conglomerated miliary tubercles, being with a caseous center and with slightly calcified areas. This condition of calcification was never observed in

any lesions of other organs. In its peripheral portion, a small number of acid-fast bacilli, epithelioid cells and giant cells were observed. In the outermost zone, fibroblasts, dense reticulum fibers were observed. The capsule of this node was thickened, adhering to the omentum. Macroscopically, scattered miliary tubercles were observed on the *serosa*, especially at the ileocaecal region. Microscopically, more minute tubercles were observed abundantly in *tunica propria* and *submucosa*, but in *tunica muscularis* and in *serosa*, though similar tubercles were observed, they were not abundant. Caseation was observed in these tubercles but slight, and a number of epithelioid cells, giant cells containing acid-fast bacilli and lymphocytes were also observed. Ulceration was not observed.

No remarkable findings were obtained in the nervous system, heart, stomach and in other organs.

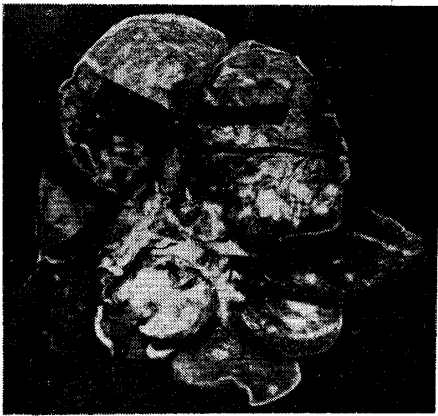


Fig. 1

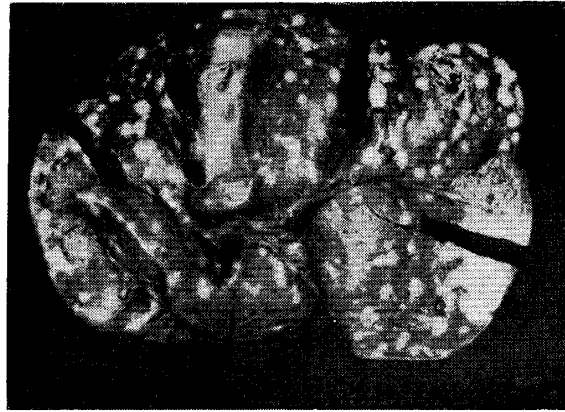


Fig. 2

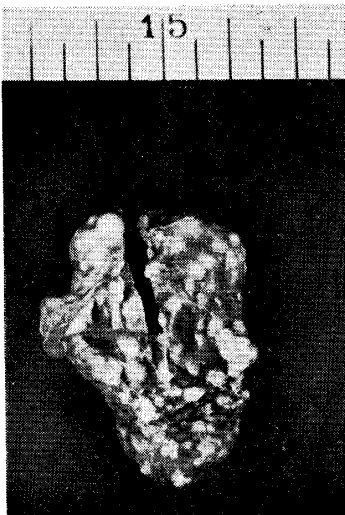


Fig. 3

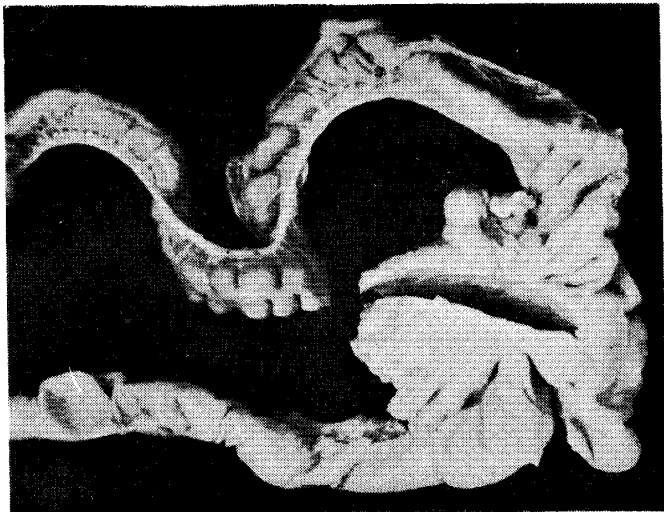


Fig. 4

- Fig. 1. Miliary tubercles in the lung.  
 Fig. 2. Miliary tubercles in the liver. Note the trace of adhesion of the left lobe to the omentum.  
 Fig. 3. Solitary and conglomerate tubercles in the spleen.  
 Fig. 4. An enlarged mesenteric lymph node,

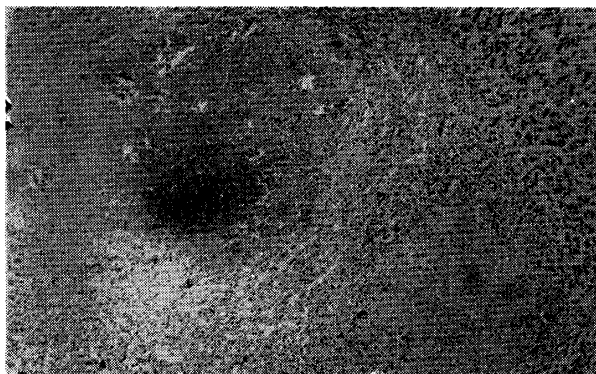


Fig. 5

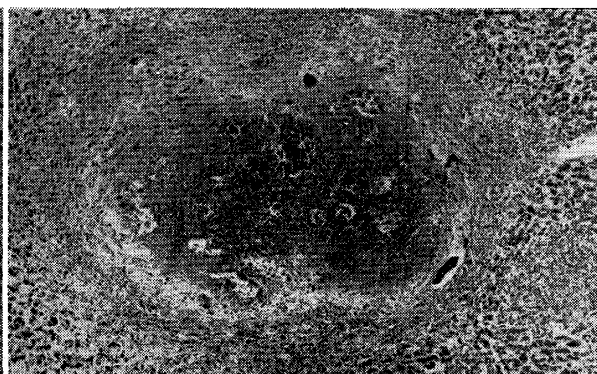


Fig. 6

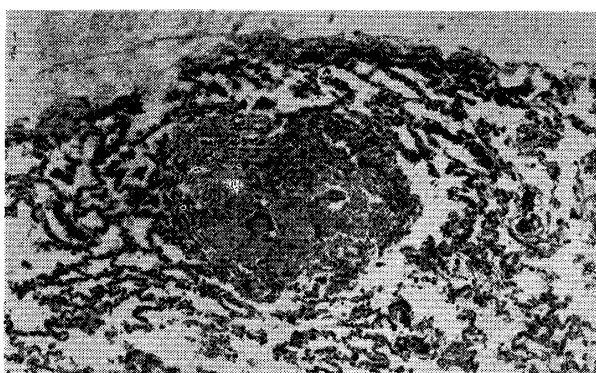


Fig. 7

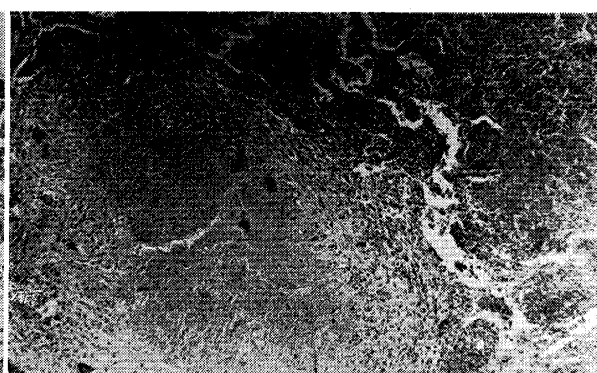


Fig. 8

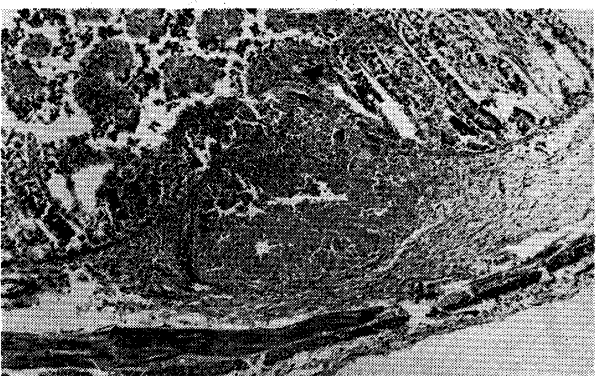


Fig. 9



Fig. 10

Fig. 5. Two miliary tubercles with a few giant cells in the liver.

Fig. 6. Two conglomerate miliary tubercles in the liver.

Fig. 7. Miliary tubercles under the pleura, consisting of epithelioid cells containing acid-fast bacilli, a Langhans' giant cell and a few lymphocytes.

Fig. 8. A miliary tubercle in the lung. Note exudative pneumonia in alveoli in the right half of the photograph.

Fig. 9. A miliary tubercle in *tunica propria* of the ileum.

Fig. 10. A miliary tubercle with giant cells in *tunica muscularis* of the ileocaecal region.

#### Isolation of the acid-fast bacilli

At autopsy, the presence of acid-fast bacilli which were suspected to be causative was proved in smears of various tuberculous materials from the carcass, and an attempt to obtain a pure culture of the organism was made applying the sulfuric acid method

to the spleen material. A portion from the spleen with lesions was ground in a sterile mortar, to which approximately 5 times its bulk of 5 per cent sulfuric acid were added to make a suspension, which was then transferred immediately into a sterile test tube and placed in the incubator, shaken at times, kept at 37°C for 20 minutes, and then centrifuged at 3,500 rpm for 20 minutes. The deposit was transferred with a loop onto the Petraghani medium and cultivation was carried on at 37°C. (Unfortunately, no culture could be obtained from this sulfuric acid treated material even after several months' cultivation). Another portion from the same spleen material was also ground in a sterile mortar, to which approximately 10 times its bulk of sterile saline (0.85 per cent) were added to make a saline suspension. With the suspension, a guinea pig (tuberculin negative) was inoculated subcutaneously in the left groin, and six weeks later sacrificed to autopsy. Four weeks after inoculation, the guinea pig was tested for the tuberculin reaction injecting intradermally with 0.1 cc of a 1:10 dilution of Old Tuberculin and gave a positive reaction (48 hours' reading, 25 by 35 mm). At autopsy, the guinea pig was found with characteristic tuberculous lesions in its various organs and lymph nodes of the body. In smears of its lesions acid-fast bacilli were detected. With the material containing necrotic lesions, the left superficial inguinal lymph node of the sacrificed animal, direct cultivation on the Petraghani medium was made, and approximately 1 month later, a pure culture of acid-fast bacillus was obtained. Various characters of the isolated organism were examined and the details will be presented below.

### Biological characters of the isolated acid-fast bacillus

#### A. Cultural.—

In order to examine the cultural characters of the isolated acid-fast bacillus, organisms grown on Petraghani medium were seeded on various artificial media shown in Table 2.

Table 2. Growth of the isolated organism (4 weeks at 37°C).

Medium	Organism				
	Isolated	Control			
		Human type H37R	Bovine type RO	Avian type AF	Avian type Takeo
Petraghani	++	++	++	.	+++
” (without glycerin)	+	+	+	.	+++
Loew.-Jens.*	++	+	+	+++	+++
” (without glycerin)	+	+	+	+++	+++
Dorset egg	±(+)	+	+	+++	+++
Kirchner (with ox serum)	+++	+++	++	+++	+++
Sauton	++(++)	++(++)	++(++)	+++	+++
Glycerin ox serum	+	+	+	.	+++
Coagulated ox serum	+	+	+	.	+++
Glycerin Potato	+	++	++	+++	+++
Glycerin agar	+	+	+	+++	+++
Nutrient agar	+	+	+	+++	+++
Glycerin broth	+	+	+	+++	+++
Nutrient broth	±(+)	+	±(+)	+++	+++
Peptone water	—	—	—	+++	+++

\* Loew.-Jens. .... Loewenstein-Jensen medium.

For parallel cultivation tests organisms from Petragnani medium cultures of *Mycobacterium tuberculosis var. hominis*, strain H37R, *M. tuberculosis var. bovis*, strain RO, and *M. avium*, strains AF and Takeo, were also seeded on the same media used for the isolated organism. Cultivation was carried on at 37°C for several weeks.

In the early period of cultivation, the growth of the isolated organism on these media was not so remarkable as compared with those of the control organisms of the human and bovine types, but in 4 to 5 weeks, it became as luxuriant as those of both types. The appearance of growth of the isolated organism on the media after 4 weeks' cultivation is briefly as follows ;

On Petragnani, Loewenstein-Jensen and both of these without glycerin, Dorset egg, and glycerin potato, the colonies were irregular, dry buff-colored and in part confluent and warty.

On coagulated ox serum with and without glycerin, the colonies were slightly moist, granular, greyish-white and thin in peripheral portions.

On the surface of liquid media, Kirchner, Sauton, and beef infusion broth with and without glycerin, a slightly buff-colored wrinkled pellicle, thick or nodular in places, occurred.

On glycerin and nutrient agar, the colonies were greyish-white or slightly buff-colored, dry, granular or in part thin ground-glass-like.

No growth occurred in peptone water.

Similar cultural characters were observed with control organisms of both human and bovine types.

On all these media the cultural reactions of control organisms of the avian type used were remarkably different from those of the isolated and control organisms of human and bovine types. The growth of the avian type was rapid and even after one week's cultivation at 37°C it was luxuriant on all media used.

#### B. Biochemical.—

Biochemical characters of the isolated organism were examined for catalase and urease activities, boiling-fastness or *Kochfestigkeit*, and the neutral red reaction. For parallel tests, organisms of the human type, strain H37R, the bovine type, strain RO, the avian type, strains 62A, AF, and Takeo, and saprophytes, strains SA-3 and SA-7, were used. The results obtained are shown in Table 3.

The catalase activity (Toda 1925a, Urabe 1933, and Hiroki 1935) of the isolated organism was less vigorous than those of the avian and saprophytic organisms. It was somewhat similar to those shown by the human and bovine organisms.

The urease activity (Toda 1925b, Hiroki 1935) of the isolated organism was positive. The organisms of the human and bovine types and SA-7 gave positive, SA-3 doubtful, and the avian type, 62A, negative activities.

The property of *Kochfestigkeit* (boil-fastness) suggested by Toda (1925b) and Hiroki (1935) was examined with the isolated and control organisms. When maintained in boiling water, saprophytic organisms, SA-3 and SA-7, were easily decolorized within a minute,

Table 3. Some biochemical activities of the isolated organism.

Strain		Catalase	Urease	Kf (min.)*	Neutral red	
Isolated		+	+	13	+	
Control	Human type	H37R	+	+	10	+
	Bovine type	RO	+	+	8	+
	Avian type	62A	++	—	5	—
		AF	++	.	5	—
		Takeo	++	.	5	—
	Saprophyte	SA-3	+++	±	1	—
		SA-7	+++	+	1	—

\* Kf.....*Kochfestigkeit* (boil-fastness); The figures indicate the maximum time in minutes taken for complete decolorization.

organisms of the avian type within 5 minutes, while organisms of the human and bovine types and the isolated took 10, 8, and 13 minutes, respectively, to complete decolorization.

The neutral red reaction for determining the virulence of tubercle bacilli first suggested by Dubos and Middlebrook (1948), reinvestigated by Morse *et al.* (1953) and Huges *et al.* (1954) was examined with the isolated and control organisms. As shown in Table 3, the isolated organism as well as organisms of the human and bovine types gave a positive reaction. While all the organisms of the avian type and saprophytes gave negative reactions.

#### Animal inoculation

To examine the virulence of the isolated organism for laboratory animals, three tuberculin-negative guinea pigs, three tuberculin-negative rabbits, and three fowls were injected subcutaneously with organisms from a young culture on Petragnani medium. Each animal receiving 5 mg wet weight of organisms ground in a sterile mortar and suspended in sterile saline (0.85 per cent), was kept for several weeks, during which time observations on changes at the site of inoculation and in neighbouring lymph nodes, changes in weight, were made and finally sacrificed to autopsy. Four weeks after inoculation, guinea pigs and rabbits were tested for tuberculin reactions, all giving positive results.

Findings in each animal observed at autopsy are shown in Table 4.

In guinea pigs subcutaneous ulceration at the site of inoculation was observed, and in various organs with the exception in the kidneys, greyish-white miliary or pin-head tubercles with or without caseation were observed. Similar conditions were observed in various lymph nodes.

In rabbits subcutaneous ulceration at the site of inoculation and a small number of tubercles were also observed chiefly in the liver, lungs and in the mesenteric lymph nodes, but in other organs and in most of the lymph nodes remarkable tuberculous lesions were not observed.



Table 4. Pathogenicity of the isolated organism for laboratory animals.

Animal		Body wt. (Kg) and sex	Tuberculin reaction*	Sacrificed after (days)	Tuberculous findings at autopsy									
					Site of inoculation	Lung	Liver	Spleen	Kidney	Inguinal l.n.**	Lumbar l.n.**	Mesenteric l.n.**	Axillary l.n.**	Cervical l.n.**
Guinea pig	No. 1	0.35M	+	46	##	##	##	##	-	##	##	+	##	+
	2	0.37F	+	39	##	##	##	##	-	##	+	+	+	+
	3	0.43M	+	44	##	##	##	+	-	##	##	##	##	+
Rabbit	No.26	2.3M	+	41	##	+	+	-	-	-	-	-	-	-
	36	2.0F	+	47	##	+	-	-	-	+	-	##	-	-
	38	1.8M	+	45	##	+	##	-	-	-	-	##	+	-
Fowl	No. 1	0.42M	.	48	-	-	-	-	-	-	-	-	-	-
	2	0.27M	.	50	-	-	-	-	-	-	-	-	-	-
	3	0.35M	.	58	-	-	-	-	-	-	-	-	-	-

\* Positive reading 10 by 10 mm or more at 24 and 48 hours after injecting intradermally with 0.1 cc of a 1:10 dilution of Old Tuberculin.

\*\* l.n. .... Lymph nodes.

In fowls tuberculous lesions were not observed.

From the results obtained through the animal inoculation experiment, the isolated organism was known to be virulent to guinea pigs, less virulent to rabbits, and avirulent to fowls, and hence known to be identical with *Mycobacterium tuberculosis var. hominis*.

### Summary and conclusions

A monkey (Kanikui-zaru, *Macaca irus*) which had been kept as a pet was autopsied and in its various organs miliary tubercles were observed. Microscopic examination proved the presence of acid-fast bacilli in smears of lesions and in histological sections. Histopathological examination further confirmed the lesions observed in the carcass were due to infection of tubercle bacilli.

A pure culture of acid-fast bacillus isolated was proved to be identical with *Mycobacterium tuberculosis var. hominis* by its biological characters and virulence to laboratory animals.

The monkey is thought to have died of tuberculosis caused by infection of tubercle bacillus of the human type.

### Acknowledgements

Our thanks are due to Mr. S. Iwata who kindly provided us with the animal for examination, to Mr. S. Okai who kindly provided us with Old Tuberculin, and to Mr. T. Aoki who kindly provided us with a culture of *Mycobacterium avium* AF.

**References**

- 1) DUBOS, R. J. and MIDDLEBROOK, G.: *Amer. Rev. Tuberc.*, 58, 698, 1948.
- 2) GRIFFITH, A. S.: *Jour. Hygiene*, 28, 198, 1929.
- 3) HIROKI, H.: *Manshu Igaku Zasshi* (in Japanese), 23, 717, 1935.
- 4) HUGES, D. E., MOSS, E. S., HOOD, M. and HENSON, M.: *Amer. Jour. Clin. Path.*, 24, 621, 1954.
- 5) MORSE, W. C., DAIL, M. C., and OLITZKY, I.: *Amer. Jour. Public Health*, 43, 36, 1953.
- 6) OKA, H.: *Kekkaku* (in Japanese), 4, 299, 1926.
- 7) RABINOWITSCH, L.: *Deutsch. Med. Woch.*, 32, 866, 1906.
- 8) SCHROEDER, C. R.: *Amer. Jour. Public Health*, 28, 469, 1938.
- 9) TODA, T.: *Nippon Biseibutsu-Byorigaku Zasshi* (in Japanese), 20, 1867, 1925a; *Ibid.*, 20, 2663, 1925b.
- 10) URABE, K.: *Nippon Biseibutsu-Byorigaku Zasshi* (in Japanese), 27, 956, 1933.
- 11) YAMAGUCHI, M., NAKAMURA, M., MISAWA, T. and YOSAWA, M.: *Nippon Juishi-kai Zasshi* (in Japanese), 7, 504, 1954.