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Investigation of the Rusty-Brown Discoloration of Silk-Fibres Caused by Microorganisms.

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With 13 plates and 2 text figures.

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Foreword.

The present article is the complete report of my work with relation to the rusty-brown discoloration of silk-fibres caused by microorganisms, on which I have been making investigations since July, 1921, at the Uyeda Silk Technical College. Some parts of this have been already published in Japanese in the Journal of the Sericultural Association of Japan (Nos. 361, 362, 363, 364, 367).

It is the writer's privilege to express his sincerest gratitude, in particular, to C. Haritsuka, Esq. Principal of the Uyeda Silk Technical College, who has given him much encouragement in his researches ; also to Prof. Dr. R. Inoue of the same college, from whom he received much valuable advice ; and, lastly, to Mr. T. Higuchi who has kindly assisted him with his experiments.

Y. Yendo.

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INTRODUCTION

Cocoons, raw silks, frisons and other waste silks often assume a conspicuous discoloration, if the processes of treatment or storage have not been properly carried out. What have been hitherto mentioned as its causes are injury by parasitic fungi during the storage of cocoons ; unsuitable water, such as that containing heavy metals in solution, employed in the filature process ; dark turbid cooking and reeling water ; adhering of the fat and oil of pupae or other dirty substances, etc. In fact, however, there exists a strange phenomenon, i, e., the rusty-brown discoloration of silk-fibres which can not be explained by the causal factors above mentioned, for instance : a) the so-called 'rusty cocoon' is produced on the straw-cocoonage when the moisture has not been properly kept out ; b) cocoons especially live ones, develop the discoloration in roasting because of being stored in piles ; c) moist frisons and boiled cocoons left over during reeling, generally turn brown gradually unless kept at law temperature ; d) raw silks are apt to become discolored when they have not dried quickly during the reeling and re-reeling process ; e) raw silks sometimes assume a brownish tinge when the process of storage has not been conducted carefully so that they absorbed much moisture ; f) frisons and other waste silks generally brown more or less in the course of the drying and storing. Again, the silk-fibres thus discolored not only lose their beautiful appearance but decrease in practical value, through loss of strength.

Indeed the reason why silk-manufacturers at great labor and inconvenience, and at enormous cost, provide various equipment for drying or storing of cocoons, as well as for filature processes, is for no other purpose than to secure a beautiful strong fibre. Hence it may be an important problem in the sphere of silk-raising, to find the real cause of the discoloration and to discover an effective method of preventing the injury. Having made experiments in the microbiological field which had hitherto not been given much attention, I have ascertained that the brown discoloration of silk-fibres may be chiefly due to the action of microorganisms. The experimental data will be described in the succeeding chapters.

Chapter I. THE DISCOLORATION OF SILK-FIBRES WITH RELATION TO BACTERIA.

A. The Discoloration of Frisons.

The frison, being produced as a waste silk in the silk reeling process, is apt to change its color considerably and such discoloration is popularly called 'oily burn' (*aburayake* in Japanese). Dr. *Inoue* and *Iwaoka* (1915) investigated this subject and expressed the opinion that yellowish discoloration is brought about by the adhering of the fat and oil of the pupa, while the brown discoloration may be due to staining by a dark brown pigment called melanin, which is produced through the action of an enzyme called tyrosinase upon tyrosin in the body of the pupa. Considering this latter view, however, we cannot accept it without question, because the tyrosinase, as a rule, loses its power of action, decomposing immediately on being boiled at 50°c., so that there is no reason to think that the enzyme still exists in the pupa's body after being boiled at 70°—80°C. for twenty or thirty minutes during the reeling process; moreover, the discoloration of frisons is not always restricted to the parts which the pupae touch, but occasionally frisons containing no pupa turn brown in the course of drying or storing. Thus we have to assume that there exists some other unknown action which causes the brown discoloration.

I have obtained in my experiments, as the causal factors, several kinds of fungi and bacteria of which I will first describe the bacteria.

The frisons first employed in my experiments were considerably browned, with a disagreeable odor, in consequence of having been stored for over ten years in the storing house of the Uyeda Waste Silk Refining Company. (PL. I. B.2.) By means of microscopical inspection, I have observed numerous bacteria, many spores of fungi and a few diatoms. In order to isolate the bacteria, a small amount of the material was put into a bouillon tube, shaken quickly to separate the bacteria, and 4 sets of agar plate culture were made

by transplanting a loopful of the bouillon into each agar medium. Thus, I have obtained as the result, 7 sorts of bacterial colonies. With these some culture experiments were made, employing such media as fresh frison, white cocoon and sericin, in order to determine whether or not these bacteria have power to cause the discoloration. The results are tabulated as follows :—

No. of colonies	Sign of colonies	Rough morphological features of colonies on agar Plate	Resuts		
			Frison	White cocoon	Sericin
1	S	round, fimbriated edged, grayish, white, dull in lustre.	+	+	+
2		round, entire, milky white, glistening, somewhat slimy.	−	−	−
3		round, yellow, dull in lustre.	−	−	−
4	M_1	roundish, grayish yellow, glistening.	+	+	+
5	M_2	amoeboid, grayish yellow, glistening.	+	+	+
6		round, transparent white, viscid.	−	−	−
7		round, grayish white, glistening.	−	−	−

Designation : + discolored, − not discolored.

As we see in the above table that, 3 kinds, i. e., S, M_1, M_2, among the 7 kinds, of bacterial colonies possess the discoloring ability. According to my comparative study, it is certain that M_1 and M_2 belong to the same species though their shapes on agar media differ from one another, consequently I will designate them M instead of M_1 and M_2 in the subsequent pages.

Regarding the discoloration of the media, the white cocoons browned so readily that they began to discolor within 24 hours at 27°C., the pigmentation gradually increasing day by day ; the frisons showed slight discoloration two days later and browned gradually thenceforward ; the discoloration of the sericin began to occur after a week beneath the bacterial colony, increasing subsequently its boundary and its intensity until it became tolerably deep brown on the whole. (PL. II. B. 2. 3.)

From the above mentioned experiments, we learn that these bacteria can produce the brown discoloration of such silky substances as cocoons, frisons and sericin.

B. The Discoloration of Boiled Cocoons left after Reeling.

It is often the experience of silk-manufacturers that boiled cocoons which have, for convenience, been left over night during the reeling operation, turn very brown and are injured in the quality of their fibres.

T. Mitani (1903) previously observed, during his experiments concerning the treatment of boiled cocoons, that injuring grade of the cocoons vary according to the methods of treatment as well as the season in which the experiments were conducted. I will here indicate the main results of his experiments.

In summer, 1) boiled cocoons, kept in a well ventilated chamber, produced deterioration with a disagreeable odor and soon turned rusty brown either partially or over the whole surface ; 2) boiled cocoons, immersed in water, somewhat deteriorated having a bad odor and showing a light yellowish brown tinge ; 3) boiled cocoons, kept in hot water of 35° C., exhibited severe deterioration accompanied by a disagreeable odor, and turned a deep brown, the water also becoming brown as gravy with fat-like pellicles floating on its surface. Next, in the autumnal experiments, deterioration and discoloration of the cocoons were far slighter in every division than in those of the summer experiments, so that almost no disagreeable odor was perceivable nor the fat-like pellicle observable at all. Lastly, in the winter experiments, no deterioration nor discoloration whatever were recognizable.

Thus we learn that intensity of the injury differs not only according to the methods of treatment, but varies by season, to express it more definitely, the injury may be quite conspicuous at a high temperature, but slight at a low temperature.

Recently *T. Watanabe* (1922), paying attention to the so-called putrefaction of the reeling water, observed numerous bacteria in it, and he, together with *Y. Chigasaki,* isolated large rod-like bacteria which can persist during high temperatures (70°—80° C. or more) of the reeling water by producing heat-enduring spores. Thus they showed that putrefaction of the reeling

water as well as boiled cocoons left after reeling is due to the action of the bacteria. In their researches, however, they have not described the discoloration of cocoons.

In order to observe the discoloration of boiled cocoons, I performed the following experiments in January, 1923, employing a race of the silkworm called *Kasuri* which was produced at the sericultural department of the Uyeda Silk Technical College in the summer of 1922.

The divisions of this experiment are as below and ten cocoons were used for each division.

Div. 1. Boiled cocoons immersed in hot water, were kept at 27° C.

Div. 2. Boiled cocoons immersed in cold water, were kept at 5° C.

Div. 3. Boiled cocoons transferred into a Petri dish, were kept at 27°C.

Div. 4. Boiled cocoons transferred into a Petri dish, were kept at 5° C.

Div. 5. Boiled cocoons dried thoroughly, were kept at 5°C.

The results of the experiments obtained after the period of 24 hours, are as follows :

Div. 1. The cocoons soaked with much water became grayish but not brown ; the water turned to turbid yellowish brown, with some bacterial pellicles floating on the surface, accompanied by a disagreeable odor.

Div. 2. The cocoons not discolored ; the water changed to a light yellowish brown, producing, however, no pellicles nor odor.

Div. 3. Full surface of the cocoons browned markedly, producing a disagreeable odor.

Div. 4. The greater numbers of the cocoons not discolored, except that some became light yellowish brown.

Div. 5. The cocoons not discolored at all.

Here microscopical observations were made of the cocoons as well as the water in each of the preceding divisions, and numerous bacteria were found in every case, especially abundantly in Div. 1 and. Div. 3. Moreover, the bacteria were isolated by means of agar plate culture, and 2 kinds of bacterial colonies obtained, S and M, which are identical with those isolated

from the discolored frisons.

From the above experiments we learn that the discoloration of cocoons occurs most severely under favourable conditions for the reproduction of bacteria ; in other words, in the case of high temperature accompanied by much moisture.

C. The Discoloration of Raw Silk.

Raw silks, reeled in the moist and hot atmosphere of reeling factories in summer, tend to lose their lustre, in the case of moist silks especially, which have been left for a long time in that condition, an evident brownish discoloration is developed. Indeed, such injuries are often actually experienced in many filature factries. I made experiments with regard to these phenomena, employing raw silks produced in the filature department of the Uyeda Silk Technical College.

The divisions of the experiments were set as follows :

Div. 1. A reel with moist raw silk was kept at a law temperature of 10°C.

Div. 2. A reel with moist raw silk was placed in an incubator saturated with dampness at 27°C.

Div. 3. A reel with moist raw silk, upon which a watery solution of the bacteria S was sprinkled, was placed in the same incubator.

The experiments were carried on from the 23rd to the 27th of January, 1923, with the following results :

Div. 1. No discoloration occurred.

Div. 2. After 2 days, turned light brown as a whole and developed brown spots here and there.

Div. 3. Began to become brown gradually from the following day.

According to the above experiments, it is evident that the raw silk may turn brown in the case of high temperature with much moisture ; moreover the discoloration may occur even in the natural raw silk, as in *Div.* 2, similarly to that inoculated artificially with the bacteria, as in *Div.* 3.

Now I will give an example with regard to the discoloration of stored raw silks. Some skeins of raw silk which had been kept in the storehouse of the Imperial Silk Company at Yokohama for nearly 2 years, from April, 1920, browned very markedly, and some of them were sent to our school for inspection. The discolored skeins were not only light brown as a whole but showed rusty-brown spots here and there, and were very rough to the touch.

I have examined them microscopically in order to determine if any microorganisms were present in them, by taking a small portion of the silk fibre on a slide-glass and rubbing off its surface with a scalpel to separate microorganisms, then tinging it with a dilute methylene blue solution. Abundant bacteria, accompanied by no fungous spore, were observed in this experiment and most of them were large and rod-shaped, 0.6—1.0μ wide by 3.5—4.0μ long, solitary or adhering two by two longitudinally.

Again, with the aim of isolating the bacteria a small amount of the discolored silk was put in a bouillon tube and incubated at 27°C. After 24 hours, the bouillon became turbid with a floating fragile pellicle at its surface. Transplanting a loopful of the bouillon into agar plate media, I have obtained 2 sorts of bacterial colonies, S and M, which are the same respectively as those previously mentioned. These bacteria were tested as to their ability to discolor silk. For this purpose, a skein of white raw silk cut into pieces nearly an inch long, was placed in Petri dishes, a small amount of water added, and the whole sterilized by means of the intermittent steaming process. The pieces were then inoculated with the bacteria S or M, and incubated at 27°C. After 3 or 4 days they showed evident brown discoloration and the red litmus paper, hung inside of the lid of the Petri dish, turned blue, indicating production of ammonia. In comparison with this, the discolored raw silk, sent from the Imperial Silk Company at Yokohama, was examined by being given some moisture in a Petri dish, and it exhibited the discoloration more deeply by and by, accompanied by strong ammonia production.

D. The Discoloration of Cocoons.

That the quality of cocoons will be injured unless the greatest precaution is used to keep them dry is a well-known fact; whereas the fact that bacteria cause discoloration of cocoons has hitherto been utterly overlooked. I have brought to light in my researches, the truth that the so-called *rusty cocoons* produced on the straw-cocoonage as well as the discolored cocoons produced during storage, except those injured by mould or those stained by excrements of silkworms, are due to the action of bacteria.

Below I will try to explain this by stating some results of experiments.

Experiment I.

In the first place, I wish to determine whether the natural cocoon alone, well moistened and at high temperature become discolored or not. Some cocoons, from which the pupae had been removed through a cutting at the side, were put into Petri dishes after being immersed for a moment in hot water at 60°C., and the dishes were then incubated at 30°C. The results observed 3 to 5 days later are tabulated as follows. (PL. III. A. B.)

No. of exp.	Kind of cocoon employed	Time of production of cocoon	Date of exp.	No. of cocoons employed	Results
1	Obkusa	Summer 1918	19, Feb. 1923	6	All turned light brown.
2	ditto	ditto	ditto	6	ditto
3	Kasuri	Summer 1922	11, Feb. 1923	14	ditto
4	ditto	ditto	14, Feb. 1923	10	All turned light brown; some developed a few yellowish spots.
5	Shokei	ditto	13, Feb. 1923	10	All turned rather dark brown.
6	Nichi 106	ditto	11, Feb. 1923	10	All turned light brown; some developed yellowish spots.
7	Nichi 105	ditto	20, Feb. 1923	8	4 turned a somewhat dark brown. the others a light brown.
8	Shinkawa-chi	ditto	20, Feb. 1923	10	All turned brown.
9	ditto*	ditto	ditto	10	All turned light brown; the discoloration lighter than the above.

10	Amoi*	ditto	ditto	10	All turned brown very slightly; 7 of them developed small pink spots here and there.

*To which some pupa decoction was added.

From these results we learn that, 1) when cocoons are left very moist in a warm place, they turn brown without any exception, although more or less variable in the intensity of the discoloration ; 2) discoloration of the cocoons may occur even though the pupa is absent ; 3) cocoons with pupa decoction added, as in the last 2 instances, discolor no more than those not so treated.

Experiment II.

In the preceding experiments, I employed only old cocoons ; I will now describe the experiments with fresh ones. The divisions of the experiments are as follows :

Div. 1. Cocoons, in a Petri dish, moistened with sterile water by spraying, to be incubated at 30°C.

Div. 2. Cocoons, in a Petri dish, moistened with sterile water by spraying and sprinkled with a little straw-dust, to be incubated at 30°C.

Div. 3. Cocoons, in a Petri dish, moistened with sterile water by spraying to be left standing in a room at a temperature of 16.5°C. to 23.5°C.

Div. 4. Cocoons without treatment, to be left in the same temperature as above.

The results of the experiments are tabulated as follows :—

Div. of exp.	Kind of cocoon employed	Date of production of cocoon	Date of commencement of exp.	No. of cocoons employed	Results
1(a)	Daishokei × Sekaiichi	11, June, 1923	13, June 1923	10	All began to turn light brown after 2 days.
1(b)	Shinshōchō	12, June 1923	14, June 1923	10	Similar to the above, but after a week most of them developed pink spots here and there.

2(a)	Daishokei × Sekaiichi	11, June 1923	13, June 1923	10	All began to turn light brown on the following day.
2(b)	Shinshōchō	12, June 1923	14, June 1923	10	Nearly the same as the above, but accompanied by same pink and yellow spots after a week.
3(a)	Daishokei × Sekaiichi	11, June 1923	13, June 1923	10	All began to turn light brown after 3 days.
3(b)	Shinshōchō	12, June 1923	14, June 1923	10	As above, but accompanied by a few pink and yellow spots after 10 days.
4(a)	Daishokei × Sekaiichi	11, June 1923	13, June 1923	10	No discoloration occurred.
4(b)	Shinshōchō	12, June 1923	14, June 1923	10	The same.

As above mentioned, cocoons soaked with moisture showed discoloration in every case and the intensity of the discoloratian became greater and greater as time lapsed, increasing rapidly at high temperature as in the divisions (1) and (2).

On the 6th day after beginning the first set (a) of these experiments, some discolored cocoons were tested by contact with moist red litmus paper. I observed that the litmus immediately turned blue, showing ammonia production.

I also made microscopical observation of the discolored cocoons, and found abundant bacteria adhering to bits of thread plucked from these cocoons, proving that the cortex of the thread is severely attacked by the parasites.

Experiment III.

Judging from the above experiments, it seems probable that natural cocoons have bacteria adhering to them from the first, for the cocoons, when placed in a moist, warm atmosphere, developed discoloration in every case in consequence of being attacked by bacteria. I therefore tried to isolate the bacteria from natural cocoons ; first, a small piece of the outside layer of the cocoon was cut off, put into a bouillon tube, and incubated to force reproduction of bacteria. After a day the bouillon became markedly turbid ; after 2 days a thick pellicle formed on the surface. Transplanting it to agar plate

cultures by the quantitative dilution method, I observed the following bacterial colonies :—

No. of exp.	Date of inspection	Cocoons employed	Bacterial colonies obtained		
			S bacillus	M bacillus	Miscellaneous bacteria
1	22. Feb. 1923	Ohkusa (summer breed produced in 1918)	⁂	+	—
2	〃	〃	⁂	—	—
3	〃	〃	⁂	—	—
4	〃	〃	+	⁂	—
5	〃	〃	⁂	⁂	—
6	〃	〃	⁂	—	+
7	〃	〃	⁂	⁂	—
8	〃	〃	⁂	—	—
9	18, June 1923	Shinshōchō (spring breed produced in June. 1923)	—	⁂	—
10	〃	〃	⁂	+	—
11	〃	〃	⁂	+	—
12	〃	〃	⁂	—	—

Designation : ⁂ many, ⁂ medium, + few, — absent.

As shown in the above table, I obtained 2 sorts of bacterial colonies, i. e., S and M, as the predominating parasites.

Experiment IV.

Some inoculation experiments are now to be described, which were made with the bacteria S and M isolated in the preceding experiments, in order to make sure whether or not cocoons inoculated with those bacteria develop the discoloration.

The cocoons employed as samples in these experiments were '*Nichi 106*,' produced in the Uyeda Silk Technical College in the summer of 1922.
4 cocoons were put in each Petri dish and sterilized by means of the intermittent steaming process ; then being inoculated with the bacteria S or M respectively, they were incubated at 30°C. on Nov. 4th, 1922.

After 24 hours all the cocoons showed simultaneously a tendency to dis-

color ; 2 days later they turned light brown and continued to increase in intensity day by day. (PL. II. A. 1. 2.) After a week, the discolored cocoons were sent to a certain reeler for reeling. He reported that it was almost impossible to reel them because of the weakness of the thread.

Experiment V.

We learned in exp. I that the discoloration of the cocoon might occur even in the case when no pupa is contained in it ; further investigation however may be required to make sure whether or not cocoons containing absolutely no oil and fat may become discolored, since there might still exist a question as to whether the discoloration be caused by the adhering of such substances. First, with the aim of removing oils and fats, some cocoons had the pupae removed by cutting one side of their outer layer, and were infused with ether in the Soxlet's apparatus for 2 days ; next, the cocoons after the ether was evaporated, were put into tubes one by one, and a small amonut of water added per each tube, sterilized by the intermittent steaming process. The bacteria S and M respectively were then inoculated and the whole incubated at 30°C. As the result, both showed the tendency to discolor from the following day and assumed an evident brownish tinge at the end of 3 days, the intensity of color increasing day by day. (PL. III. C, 2, 3.)

E. Source of the Bacteria and Course of their Infection.

As I have shown in the preceding pages, the cause of the rusty brown discoloration of cocoons, raw silks, frisons, etc., may be recognized as the action of the bacteria S or M.

Now considering the source of the bacteria, the fact has already been shown that they are found in abundance adhering to the natural cocoons, hence we may anticipate that the germs of the bacteria will be found to come either from the air of the cocoonery or from the straw to which the cocoons are attached.

I believe, after having conducted experiments for the detection of the

bacteria, that the main source is the straw rather than the air, although the latter of course may be an accessory source. The data of the experiments are described below.

(a) Isolation of bacteria from the straw.

A small piece of straw was put into a bouillon tube and incubated at 30°C. When observation was made on the following day, the fluid was turbid with a thick pellicle on the surface. Then agar plate cultures were made through the ordinary process of dilution, with the following results :—

Date of exp.	No. of exp.	Bacterial colonies obtained		
		S bacillus	M bacillus	Miscellaneous bacteria
19. Jan. 1923	1	+	##	−
//	2	##	−	−
//	3	##	−	−
26. Jan. 1923	4	##	+	−
//	5	##	##	−
//	6	##	##	+
//	7	##	##	+

Thus I obtained S and M as the predominating bacteria. These bacterial colonies were respectively compared through cultural researches with those which had been obtained formerly from the white cocoons or frisons, and the fact confirmed that they belonged respectively to the same species. (PL. III. D. 4. 5.)

(b) Isolation of bacteria from the air of the silkworm rearing-room and the cocoonery.

I made a microbiological examination of the air of a certain rearing room of the Uyeda Silk Technical College during 10 days from the 4th to the 13th of June, 1923. 4 Petri dishes containing an alkaline agar medium were employed each day, the duration of the exposure being the same in each case. i. e., 5 minutes at noon, followed by incubation at 25°C. The results are tabulated as follows :—

Date	No. of exp.	No. of bacterial colonies developed			
		No. of S bacillus	No. of M bacillus	No. of miscellaneous bacteria	Total number
4th	1	1	1	1	3
//	2	0	0	6	6
//	3	0	0	7	7
//	4	0	1	3	4
5th	5	1	1	0	2
//	6	0	0	2	2
//	7	0	0	0	0
//	8	0	0	2	2
6th	9	0	2	3	5
//	10	0	0	7	7
//	11	0	0	4	4
//	12	0	0	4	4
7th	13 *	...	...	...	...
//	14	0	0	3	3
//	15	0	1	0	1
//	16	0	0	4	4
8th	17	0	0	10	10
//	18	0	2	1	3
//	19	0	0	10	10
//	20	0	0	9	9
9th	21	0	0	6	6
//	22	1	0	0	1
//	23	0	0	14	14
//	24	0	1	3	4
10th	25	2	1	0	3
//	26	2	0	0	2
//	27	0	0	3	3
//	28	4	1	0	5
11th	29	0	0	22	22
//	30	0	0	13	13
//	31	0	0	18	18
//	32	0	0	18	18
12th	33	0	0	2	2
//	34	1	0	3	4
//	35	0	0	3	3
//	36	0	0	4	4
13th	37	0	0	12	12
//	38	0	0	6	6
//	39	0	0	6	6
//	40	0	1	7	8
Sum		12 :	12 :	216 :	240

* The dish was broken.

That is, the number of both S and M colonies respectively is just 5% of the total number of the bacterial colonies.

(c) Some experiments as to the heat resisting power of the bacteria.

The fact that bacteria, parasitic on cocoons, can persist during the high temperatures of the drying process (generally 70°C.—80°C. for 8 hours), and moreover being transmitted to raw silks, frisons, etc., can endure the temperature both of the boiling water (which is kept at the boiling point for a short time) and the reeling water (65°—82°C.), is due to the heat resisting spores produced readily by these bacteria. I will now describe my experiments with regard to the heat resisting power of the bacterial spores.

(*1*) *Resistance to dry heat.* As to the method of the experiment, first some of the bacteria were smeared on a sterilized cover-glass and placed in a Petri dish, then introduced into a hot-air sterilizing apparatus. Then after being heating up to the required degree for a certain time, the cover-glass was put into a bouillon tube and incubated at 30°C. for 2 days. The results obtained are here tabulated ;

Temperature (C) applied	Duration of time	Results	
		S bacillus	M bacillus
98°	3 hours	developed	developed
98°	4 //	//	//
120°	30 minutes	//	//
125°	//	//	//
130°	//	not developed	not developed
135°	//	//	//

From the above table, we learn that both species of bacteria can withstand dry-heat up to 125°C. for 30 minutes, and are destroyed at a temperature above 130°C. continued for 30 minutes.

It was determined by Chigasaki's researches that the bacteria which injure cocoons (probably identical with S bacillus) could withstand the action of dry heat at 120°C. for 5 hours, but were killed at the same temperature after 7 hours, and also at 150°C. in 30 minutes, at 170°C. in 10 minutes,

or at 130°C. in 1 hour.

(2) *Resistance to moist heat.* As to the method of this experiment, some of the bacteria, containing spores, were transferred into a bouillon tube heated up to the temperature and kept at that temperature during a certain length of time by means of the water-bath apparatus. Afterward the bouillon tube was iucubated at 30°C. for 2 days and then an inspection was made to see whether or not the bacteria had developed. The following results were obtained :

Temperature (C) applied	Duration of time	Results	
		S bacillus	M bacillus
85°	1 hour	developed	developed
90°	1 //	//	//
93°	1 //	developed or killed	//
95°	1 //	//	developed or killed
98°	20 minutes	developed	developed
98°	40 //	//	killed
98°	1 hour	developed or killed	//
98°	1 h 20 m	killed	//

According to the above table, S bacillus withstands the action of boiling water at approximately 100°C. for from 40 mimutes to 1 hour, and M bacillus persists during nearly 1 hour at from 93° to 95°C. or at approximately 100°C. for 20 minutes.

I may add here that the cocoon deteriorating bacteria investigated by Chigasaki, were destroyed by being exposed for 1 hour at the temperature of boiling water.

From these above mentioned experiments, it will be evident that the bacteria S and M, on account of possessing heat resisting power, are never destroyed by being exposed to a very high temperature during the processes of drying and reeling the cocoon.

(d) Germs of the bacteria in the boiling and reeling water.

I investigated the bacteria existing in the boiling and reeling-water,

although many demonstrations in this line had already been made by *Chigasaki* and *Watanabe*. In my experiments, ordinary agar plate cultures were used, two loopfuls each of the platinum loop, being taken from the reeling-water of the machine-reeling factory of the Uyeda Silk Technical College.

Date of experiment	No of exp.	No. of bacterial colonies obtained			
		in boiling water		in reeling water	
		S bacillus	M bacillus	S bacillus	M bacillus
Jan. 17th. 1923	1	0	1	0	many
//	2	0	0	0	0
//	3	0	0	0	0
//	4	0	0	0	0
//	5	1	0	0	0
//	6	0	0	1	0
Jan. 18th. 1923	7	0	0	1	0
//	8	0	0	0	many
//	9	0	1	0	0
//	10	0	0	1	1
Jan. 19th. 1923	11	...	...	3	1
//	12	...	...	4	0
//	13	...	...	0	0
//	14	...	...	0	0

The experiments tabulated above were made with boiling water used only once, and with reeling water used only one hour. I also isolated bacteria from boiling water used twice and from reeling water used for 2 hours, in order to compare the number of the bacteria.

Date of experiments	No. of exp.	No. of bacterial colonies obtained			
		in boiling water		in reeling water	
		S bacillus	M bacillus	S bacillus	M bacillus
Jan. 18. 1923	1	4	4	17	many
	2	3	1	21	11
	3	2	1	26	7
	4	3	1	many	many

From the above tables, we learn that: 1) both the boiling water and the reeling water contain 2 kinds of bacteria, S and M; 2) The numbers of the bacteria are greater in the reeling water than in the boiling water, and greater in the older boiling or reeling water than in the fresher water.

These results agree, in the essential points, with those obtained by *Chigasaki* and *Watanabe* who did not, however, report on the M bacillus obtained in my experiments. At any rate, it is certain that the bacteria are present even in rather fresh reeling and boiling water and that they are present in enormous numbers in staler water, so that they may adher to the raw silks or frisons, to cause later the brown discoloration.

F. Reason of the Discoloration.

It has been shown, through my experiments mentioned in the foregoing pages, that the rusty-brown discoloration of silk-fibers may be due to the action of the bacteria S and M. If so, we must show further how this takes place.

Silk-fibres consist of two brins, whose core is a bunch of numerous and extremely thin fibrils composed of fibroin, the bunch being coated densely with sericin. Both fibroin and sericin are proteid materials, containing nitrogen as one of their components, and in the course of their decomposition they are converted into peptone at first, then into several amides, lastly producing ammonia gas. Therefore, if the bacteria feed upon the silk-fibre, decomposing its substance, some ammonia gas must be produced as a final product. In order to confirm this conclusion, I cultivated the bacteria S and M with the media of raw silk, egg white and peptone water.

(*1*) *Culture with raw silk.* The bacteria S and M, removed from agar slant cultures, were inoculated respectively into each of the culture media and incubated at 27°C., a red litmus paper being hung inside the lid of each Petri dish.

I observed, on the following day, that all the test papers began to show change of color accompanied by the peculiar odor indicating production of

ammonia.

(2) *Culture with egg-white.* This culture medium was prepared by separating egg-white, with all possible aseptic precautions, into disinfected test-tubes where it was coagulated and sterilized by the steaming process for 30 minutes on each of 3 successive days. The bacteria were inoculated into the media and a red litmus paper was hung from the top of each tube.

On the following day, it was observed in both S and M that the test-paper began to change blue showing production of ammonia and the color became deeper and deeper all the time ; the medium also changed color becoming yellowish brown on its upper surface after the first 2 days and the area and intensity of the pigmentation increased gradually. Moreover it subsequently became translucent showing peptonization of the egg-albumen. (PL. II. C. 2. 3.) M was somewhat inferior to S in its action.

(3) *Culture with Dunham's peptone solution.* Each test-tube contained the peptone solution, was inoculated with the bacteria S or M, also had red litmus paper hanging from its top and was incubated at 27°C.

The results were that, in S, the test paper began to change into blue on the following day, and the solution clouded markedly with a pellicle on its surface after 3 days ; in M, similar changes were delayed nearly one day. After a week, in both cases, evident presence of ammonia was shown on the test of Nessler's reagent, abundant yellow precipitates being produced.

Considering the results of the 3 sets of experiments above mentioned, we may assume that the bacteria first produce peptone, acting upon such protein substances as silk-fibre or egg-white, and then cause the separation of ammonia from them.

According to chemistry, as a rule, some amino acids are derived, as an intermediate product, in the course of the decomposition of protein into ammonia gas ; and tyrosin, one of the amino acids, is readily changed into a dark brown pigment named melanin, through oxidation by an enzyme called tyrosinase.

The reason, therefore, of the brown discoloration of silk-fibres is discovered if we can prove tyrosinase to be present in the bacteria.

I tried to demonstrate this, the experiments and the results being as follows :—

(*1*) *Cutlure with bouillon media to which some tyrosin was added.* Having been incubated at 30°C., S bacillus showed vigorous growth, first causing the solution to become clouded and forming a thick pellicle on the surface afterward changing the color to a deep brown indicating the presence of tyrosinase ; M bacillus also developed fairly well, this solution showing some discoloration in consequence of the action of the enzyme, but in less degree than the former.

(2) *Culture with agar slant media to which some tyrosin was added.* Having been incubated at 30°C., S bacillus spread over nearly the whole surface of the slant within 2 days, discoloration of the medium began to occur beneath the bacterial growth after 3 days, deepening and spreading until at last it affected the whole of the medium ; M bacillus also developed very rapidly, and began to cause gradual brown discoloration of the medium from the fifth day. (PL. II. D. 2. 3.)

As the above experiments show, both the bacteria, S as well as M, possess ability to change tyrosin into a brown pigment by secreting tyrosinase. We may thus conclude from the demonstrations described in the foregoing pages, that the discoloration of silk-fibres is due to the production of a brown pigment called melanin through the oxidation of tyrosin by tyrosinase which is found in the bacteria, and that the tyrosin is derived from decomposition of protein substances such as silk-fibre.

The next question which comes under our consideration is whether fibroin or sericin (of these the silk-fibre is composed) is more readily discolored. I have already observed that cocoons, containing much sericin, became discolored more deeply than raw silks or frisons, which had lost a large portion of sericin in the reeling water. For difinite comparison, I made cultures employing pure sericin and pure fibroin media.

Remarks : 1) Colloidal solution of sericin was obtained from the silk layers of white cocoons, by dipping them into water, heating them in an autoclav for 30 minutes under double atmospheric pres-

sure, and then the solution obtained slowly boiling down.

(2) Fibroin fibre, separated from sericin, was used either just as it was, or as a colloid, the latter being obtained by dissolving in a solution of caustic potassium, precipitating again by concentrated hydrochloric acid, and then washing thoroughly with water.

The results obtained in these culture experments showed that, in the sericin media, both S and M bacillus began to cause discoloration from the upper surface of the medium within one week, and afterward the whole medium turned dark brown ; their appearances 3 weeks later are shown in PL. II. B. 2. 3. In the fibroin media, however, neither the fibrous nor the colloidal formes exhibited recognizable growth of the bacteria for 3 weeks or more, and consequently there was almost no discoloration.

It is thus evident that sericin is discolored far more easily than fibroin.

G. Character of the Discolored Silk-Fibre.

The pigment produced by bacteria, which discolors silk-fibre is a substance called melanin as already mentioned. This substance dissolves more or less readily in hot water, so that the discolored silk-fibre may be decolorized to some extent by a refining process. But in case of severely discolored silk-fibres, the pigment is so throughly absorbed in the fibre that it is difficult to remove it completely.

Therefore, when textiles are manufactured of such discolored silk-fibres, it is impcsible to make them look well. Not only is their appearance spoiled but such textiles are not durable, the fibre having been weakened by the action of the parasites. It is often found in actual reeling that the silk of the rusty cocoon produced in a very moist hot atmcsphere is difficult to unwind and breaks frequently. I have confirmed this experience when working with the cocoons purposely discolored by inoculation of the bacteria.

Let us now consider the strength of discolored raw silks. The material employed in this experiment was raw silk of 14 denier, which was cut into 4-foot lengths. These were wound, each one, around a piece of thick paper,

then placed in Petri dishes and sterilized by the intermittent steaming process. Three divisions were made for the experiment, i, e., 1) control, 2) inoculated with S bacillus, 3) inoculated with M bacillus. All were incubated at 27°C. and examined after a week had passed.

At the beginning of the test, all the pieces of thick paper together with the raw silk were dried thoroughly in order to render the water content equal. The serimeter was then used to measure their tenacity and elasticity ; the following figures are the average of 40 tests.

Division of experiment	Tenacity (mg)	Proportion of tenacity to the control	Elasticity (mm)	Proportion of elasticity to the control
1) control	38,8	100,0	173,6	100,0
2) inoculated S	33,6	86,6	126,6	73,0
3) inoculated M	36,6	94,3	135,0	77,7

From the above table we learn that damage is the most severe in Div. (2), showing decrease of 13.4% in tenacity, and of 27.0% in elasticity ; and Div. (3) is next, decreasing 5.7% in tenacity and 22.3% in elasticity. Since such great damage results within a week, it may be inferred how severe the injury must be when the process continues for a longer time.

Materials for silk spinning such as frisons, floss, *'lost-thread-cocoons'* and other waste silks are often discolored by these parasites, at the same time having their quality injured, and their strength decreased, resulting in much loss during the spinning, and still more during the dressing operation. It is needless to say, moreover, that textiles made of such imperfect materials are inferior not only in appearance but in durability.

Chapter II. THE SILK DISCOLORING BACTERIA.

As I have stated in the preceding chapter, there are two kinds of bacteria, S and M, which cause the discoloration of silk-fibres. I will now describe their morphology, cultural features, physiological features, classification, etc.

A. S Bacillus.

(a) Morphology.

(*1*) *Vegetative cells.* Large, rod-shaped, with rounded ends ; in agar slant culture, 81.5— 4.5μ 8 long 4 and 0.9—1.2μ wide, 8 commonly 3.0μ × 1.0μ ; in condensed water also, nearly the same size ; size of the bacillus not markedly variable under different culture temperatures, but more or less varying in form and size with varying culture media, for example, in nitrate peptone solution showing tendency to become slender, i. e., 2.5—5.0μ long, 0.8—1.0μ wide, commonly 3.7 × 0.85μ ; usually solitary, but occasionally appearing with a septum across the middle showing a phase of division moreover at intervals accompanied by short or long chains, and especially, in nitrate peptone media, the filaments sometimes appearing to branch (pseudobranching). (PL. V. 1. 2.)

(*2*) *Sporangium.* Formed already after 24 hours at 27°C. on agar or potato medium ; rod-shaped, almost the same as the vegetable cells, commonly 3.7μ long, 0.9μ wide ; solitary or in chains. Endospores in the centre or nearly s . (PL. V. 3.)

(*3*) *Endospores.* Elliptical, 1.6μ long, 0.8μ wide, thick-walled, glistening, not adherent to the wall, when mature separating readily from the sporangium ; germination equatorial.

(*4*) *Motility and flagella.* Actively motile in bouillon and condensed water of agar slant culture ; flagella, 5—7 in number per rod, peritrichiate, stained well by Bunge's as well as Löeffler's method. (PL. V. 4.)

(*5*) *Capsules.* Present but thin.

(*6*) *Pseudozooglea.* Produced after 2 days at 27°C. on the surface of bouillon as well as other liquid media ; grayish white, brittle, heavy ; sink down immediately when shaken.

(*7*) *Involution forms.* Present on acid media with variable irregular outline, generally extremely distended, containing large vacuoles ; the faculty of forming spores absent.

(*8*) *Staining reactions.* Gram positive ; stained readily by gentian violet, carbol fuchsin and Löeffler's alkaline methylene blue.

(b) Cultural features

(*1*) *Agar stroke.* After 24 hours at 27°C., developed as a broad, moist white layer along the streak line, its edge fimbriate ; condensed water somewhat clouded. After 2 days, growth thickened, spreading 1*cm.* wide, grayish with dull lustre ; medium browned 2 weeks later. (PL. V. 5. 6. 7.)

(*2*) *Potato.* After a day at 27°C. faintly developed ; after 2 days forming a grayish white layer, surface flat, dull in lustre and brittle in consistency. When older, the bacterial layer turned light yellowish brown and the medium dark brown ; ammoniacal odor produced, changing the red litmus paper hung over the medium into blue. (PL. IV. 6.)

(*3*) *Agar stab.* Surface growth grayish white, dull in lustre ; line of puncture filiform, villous or plumose. (PL. V. 6. 7.)

(*4*) *Gelatin stab.* After 20 hours at 19°C. begun to liquefy in infundibuliform ; after 48 hours, upper region liquefied nearly 5 *mm.* deep in stratiform ; line of puncture liquefied in sac-form, sending plumose or arborescent outgrowth into surrounding medium. The liquefied part was clouded with flocculent or dusty sediments ; whitish pellicles sometimes floated on its surface ; reaction of liquid alkaline ; red litmus paper hung from top of tube turned blue in consequence of production of ammonia.

(*5*) *Glucose added gelatin stab.* After 20 hours at 19°C. liquefied in crateriform around the stab point ; line of puncture filiform or beaded ; no liquefaction occurred. After 48 hours, upper region of medium liquefied nearly 3 *mm.* deep in stratiform, indicating acid reaction but no gas. After

3 days, abundant minute, punctiformed, free colonies appeared scattering in the medium.

(*6*) *Nutrient broth.* After a day at 27°C. fluid clouded with scant sediment; after 2 days formed heavy, grayish white pellicles or ring at the surface. The pellicles or ring readily broke into fragments when shaken and sank down as sediment; again when shaken more quickly the sediment scattered making a homogenous turbid fluid. After a week, the fluid became rather clear, sediment increasing at the bottom.

(*7*) *Milk.* After a day at 27°C. coagulation of casein occurred succeeded by peptonization; after 2 days, greater part of coagulum peptonized, showing fair acid reaction.

(*8*) *Litmus milk.* After a day at 27°C., coagulated, then peptonized, showing acid reaction; after 2 days, litmus reduced and the whole turned somewhat yellowish brown.

(*9*) *Gelatin colonies.* After 2 days at 19°C. colonies were visible macroscopically, while liquefaction of medium began to occur on the next day. Surface colonies grayish white, usually irregularly round, somewhat raised, surface rough, edge fimbriate or curled with slender outgrowths; in older colonies, surface irregularly wrinkled (PL. IV. 4a.) and some with fin-like outgrowth at margin. (PL. IV. 5b.) Liquefaction saucer-like; liquid not turbid.

Buried colonies small, irregular, grayish white, possessing slender root-like outgrowths around their surface. (PL. IV. 4b.)

(*10*) *Agar colonies.* Growth very rapid at 26°C.; after a day, developed to a tolerable size. Surface colonies commonly roundish, thick, grayish white, surface rough, dull in lustre. (PL. IV. 3a.)

Buried colonies rather mycelioid with filamentous or fimbriate edge. Bottom colonies resemble buried ones on the whole but thinner. (PL. IV. 3b.)

Size of colonies on agar plate culture commonly 3—4*mm.* in diameter (PL.IV. 1.), when densely sown very small and when thinly sown become so large as to attain to 2*cm.* in diameter. (PL. IV. 2.)

(c) Physiology

(*1*) *Ammonia production.* Culture media containing several nitrogen. compounds were employed for testing. I have used, as a method of detection, red litmus paper hung from the top of tube, and also Nessler's reagent; the results of the experiments are tabulated as follows :—

Culture media	Turning blue of red litmus paper			Nessler's reaction
	after 1 day	after 2 days	after 3 days	
Uschinsky's solution	−	−	−	+
Cohn's solution	−	−	−	− (?)
Urea solution	++	+++	+++	+++
Winogradski's solution	+	+	++	++
Egg-white	+	+	+	...
Peptone water	+	++	++	++

Note: 1) Cultivated at 27°C.

2) Urea solution was prepared with $100_{cc.}$ of soil extract, $0.05_{gr.}$ of K_2HPO_4, and $5_{gr.}$ of urea.

3) Designation: − no reaction, + feeble, ++ moderate, +++ strong.

4) Nessler's reaction was examined with ten days' culture.

Erom the above results, we see that the bacillus is able to produce ammonia by decomposing the protein of egg-white, peptone, asparagin in Uschinsky's medium, ammonia sulphate in Winogradski's medium, as well as Urea.

(*2*) *Nitrite production.* Ten days' culture with ordinary peptone water exhibited no reaction on use of Griess' reagent, while that containing a little potassium nitrate gave fairly red reaction, showing production of nitrite.

(*3*) *Temperature relations.* Agar slant media, inoculated with S bacillus, were exposed for 48 hours at several different temperatures in order to see the relation of temperature to growth.

Temperature (C) applied.	State of growth.
0°	−

10°	—
14°	+
21°	+
25°	++
29°	++
35°	++
37°	+++
40°	+++
44°	++
47°	+

According to the above table, the bacillus exhibits almost no development at a temperature below 10°C., development increases with increasing temperature above 14° C., and optimum temperature for growth lies between 37° and 40°C.; growth does not stop even at 47°C., so that the bacillus is to be included in the group of thermophiles.

(4) *Resistance to sun-light.* By exposing to sunlight for certain periods of time, bacteria which had been sown evenly in agar media on Petri dishes, I ascertaind that 3 hours' exposure did not destroy many of the bacilli; after 5 hours of exposure the number of bacterial colonies was very much decreased, but even after 8 hours they were not killed entirely.

(5) *Indol production.* No reaction was observable with Salkowsky's method of detection, employing bouillon culture for 1 week, 2 weeks and 3 weeks duration.

(6) *SH_2 production.* In milk culture at 27°C., the lead-paper hung from the top of the tube turned dark brown at its lower end after 3 days and the reaction increased day by day, showing vigorous production of hydrogen sulphide. In broth culture also, the lead-paper began to turn dark brown after 2 days.

(7) *Relation to oxygen.* Anaerobic cultures with Buchner's method showed that very poor growth occurred on agar slant after one day but later there was no visible development. After 23 days had passed, the plug of the tube was removed to introduce air; it suddenly began to grow, and thenceforward developed copiously, forming a thick, broad layer. The

bacillus is therefore to be assigned to the group of aerobic organisms.

(*8*) *Resistance to acid and alkali.* The bacillus was cultivated in broth media prepared by titrating neutral broth with standard hydrochloric acid or caustic potash solution and gave several reactions.

Note : 1) In the following table, degrees of reaction are shown by the volume (cc.) of the standard acid or alkaline solution which was needed to titrate one liter of the neutral broth.

2) Cultivated at 27°C. and inspected 5 days later.

Reaction of culture media		Growth	Pellicle
acid	40°	−	absent
//	30°	−	//
//	20°	++	//
//	10°	++	present
neutral	0°	+++	//
alkaline	10°	+++	//
//	20°	++	//
//	30°	++	//
//	40°	++	absent
//	50°	+	//
//	60°	−	//

According to the above table, the optimum reaction for growth lies between alkaline 10° and neutral ; and growth decreases in proportion as reaction is apart from the above range, until finally no growth is to be seen above acid 30° and alkaline 60°.

(*9*) *Enzyme.*

Note : 1) The following experiments on the enzymes are preliminaries which want further research.

2) In every experiment a control tube was prepared for comparing the reactions.

Diastase. The bacillus was cultivated with a kind of starch media which was prepared by boiling nitrate peptone solution containing some potato-starch. After one day at 27°C. the fluid clouded and produced a pel-

licle ; 6 days later invert sugar was detected, showing red precipitates, that is, the presence of diastase, on application of Fehling's solution.

Invertase. Culture with cane-sugar added to broth showed a feeble clouding after one day at 27°C., and some bottom precipitates were produced after 2 days but no pellicle, nor any fermentation. After 5 days, presence of invertase was tested by Fehlings' solution, giving positive result.

Lipase. Culture with nitrate peptone solution to which a small amount of oil of the pupa was added, being alkaline at first showed neutral reaction after one day and became slightly acid after 5 days, and continued to increase in acidity. This is due to the production of fatty acid derived from the oil through the action of lipase in the bacteria.

Oxidase. No reaction was exhibited by testing broth cultures with guaiacum tincture.

Peroxidase. Blue reaction appeared on testing broth cultures with guaiacum tincture together with hydrogen peroxide ; also red reaction, from guaiacol together with hydrogen peroxide, showing the presence of peroxidase.

Tyrosinase. we have already learned, in the previous chapter, that positive results were obtained when cultivating with tyrosin added to broth, as well as with tyrosin added to agar media.

Proteolytic enzyme. Egg-white was peptonized ; gelatin medium liquefied quickly ; casein of milk also peptonized.

(*10*) *Acid production.* Positive results were obtained by testing with blue litmus paper in broth culture containing cane-sugar, after 5 days at 27°C. ; in broth culture containing glycerin, after 4 days at the same temperature ; in broth culture containing lactose after 6 days at the same temperature.

(d) Identification of species

S bacillus is closely allied to Bucillus subtilis *F. Cohn* as well as Bacillus megatherium *de Bary.* They are compared in the following table :—

	Bac. subtilis	Bac. megatherium	S bacillius
Agar stroke	Growth abundant, spreading, glistening, dirty gray, edge entire, sometimes rugose at surface; condensed water turbid with cloudy gray precipitates.	No remarkable difference.	Almost the same, but medium browned in older culture.
Agar stab	Growth good, roundish, spreading; line of puncture filiform.	Almost the same.	Line of puncture villous or plumose.
Potato	Growth dirty white to yellowish, with wavy edge, somewhat raised, dull in lustre, later assuming floury appearance.	Similar but the color usually more yellow.	At first, grayish white as B. subtilis, later assumes light yellowsh brown.
Gelatin stab	Growth whitish gray; liquefaction crateriform to cylindrical with a thick white pellicle on the surface; no remarkable liquefaction occurs along the line of puncture.	Liquefaction saccate around the line of puncture.	Liquefaction first infundibulor, later saccate, often sending plumose or arborescent outgrowth into the surrounding medium; pellicle is occasionally formed.
Broth	Turbid, with a pellicle and some precipitates.	Almost the same; pellicle is sometimes formed.	No remarkable difference.
Ammonia	Readily producible from several media.	.....	Same as Bac. subtilis.
Size of vegetative cells	$1.2-3\mu \times 80.-1.2\mu$	$1.6-5\mu \times 0.6-0.8\mu$	$1.5-4.5\mu \times 0.9-1.2\mu$
Flagella	Peritrichiate.	The same	The same.
Spores	Elliptical, $1-2\mu \times 0.6\mu$	.....	Elliptical, $1.6 \times 0.8\mu$
Indol	.....	Negative.	The same.
SH_2	.....	Produced abundantly.	The same.
Agar colonies	Surface colonies: irregularly formed throughout, seldom entire edged, usually fimbriate or curled, central part yellowish, finely granular. Buried colonies: resemble the above but more compact, thicker and opaque, branches irregular and knotted.	Surface colonies: in young stage, have thin extremely transparent zone around the initial colonies, which soon become opaque, coarse crumbly, brownish yellow, usually with anastomosing branches at edge. Buried colonies: small irregularly formed, en-	Both the surface and buried colonies closely resemble those of Bac. subtilis.

		tire edged, opaque, compact, possessing fine outgrowths around periphery.	
Gelatin colonies	Surface colonies: roundish or irregularly formed, granular, yellowish to brownish in the central part, with wavy or curled edge. Buried colonies: small roundish, entire edged, crumbly, yellowish to brownish, occasionally with fine hairy outgrowths	Surface colonies: gray white, transparent, middle part finely or coarsely granular, surrounded with fine hairy outgrowth at periphery. Buried colonies: resemble the former but smaller and more compact.	Both the surface and buried colonies resemble those of Bac. subtilis rather than Bac. megatherium.

As the above table shows, S bacillus and Bac. subtilis closely resemble one another in important features, such as agar colonies, gelatin colonies, potato culture, etc, ; there may, however, be found some differences in gelatin stab, agar stab, etc.

With both S bacillus and Bac. megatherium, liquefaction of gelatin stab is saccate but they differ more or less in many other features, We, therefore, judge that S bacillus is allied rather to Bac. subtilis. Again, having compared S bacillus to the bacteria inimical to boiled cocoons found in reeling water by *Watanabe* and *Chigasaki*, I believe that they are identical, since they show identical features whether in agar stroke, potato, gelatin stab, broth with glucose, milk, or litmus milk ; they are the same also in spore production, in indol reaction, in reaction to Gram's stain, in resistance to heat, etc. and in size of vegetative cells ; the size of cells of the latter being 2.5—4.5μ × 1.0—1.5μ.

B. M Bacillus.

(a) Morphology

(1) *Vegetative cells.* Rod-shaped with rounded ends ; those developed on agar slant, at a high temperature of 30°C., 1.8μ—4.5μ long by 0.6μ wide, commonly 3.2μ × 0.6μ, but those developed at a low temperature of 24°C.,

attaining a size of only 1.0μ—4.0μ long, 0.5μ wide, commonly 2.6μ × 0.5μ ; usually solitary, but sometimes in chains of two or more.

(2) *Sporangia.* Produced on agar media after 3 days at 28°C. ; oval or elliptical, commonly 2.4μ × 1.1μ being shorter and thicker than vegetative cells ; often adhering two by two longitudinally. Endospores are produced, situation central or excentral.

(3) *Endospores.* Elliptical, 1.25μ × 0.75μ ; thick-walled, glistening ; when matured separate from the sporangia ; difficult to stain with carbol fuchsin.

(4) *Motility and flagella.* Actively motile in fresh broth culture, or condensed water of agar slant culture. Flagella peritrichiate, 4—8 in number per individual, their length being 3—5 times of the length of the body.

(5) *Capsules.* Older cultures produce much slime which can be drawn into threads more or less by platinum needle ; a well-defined capsule is made visible by the common staining method.

(6) *Zoogloea.* Present at the surface of such liquid media as broth ; grayish yellow, membranaceous, not readilybroken up, nor sunk by shaking.

(7) *Involution forms.* Club-shaped or curved forms are to be seen in older cultures.

(8) *Staining reactions.* The bacillus is not retentive of Gram's stain but is stained with carbol fuchsin, methylene blue, and gentian violet, in both aqueous and alcoholic solutions.

(b) Cultural features

(1) *Agar stroke.* After 1 day at 27°C., a thin but broad bacterial layer developed along the streak line ; condensed water became very turbid ; after 2 days, thickness of the growth increased, appearing grayish yellow, glistening, butyrous. The lower portion of the growth is wider than the upper portion ; margin irregularly lobed or lacerate. The medium became brown from the upper part after nearly 2 weeks. (PL. VII. 4. 5. 6.)

(2) *Potato.* After 20 hours at 27°C., a yellowish slimy growth appeared on a wide area of the medium, soon spreading over the full surface,

becoming opaque and membranaceous with small folds. After 48 hours, the growth increased in thickness showing extremely irregular anastomosing folds, and the color turned to yellowish brown ; sometimes minute grayish white spots were produced over the whole surface.

(*3*) *Agar stab.* After 1 day at 27°C., surface growth copiously developed, grayish white with lobate or lacerate edge ; while growth along the line of puncture was scanty, not spreading into the surrounding medium.

(*4*) *Gelatin stab.* After 1 day at 19°C , liquefaction occurred in crateriform around the puncture ; after 2 days, 5*mm* of the upper portion was liquefied in stratiform, and the liquid clouded homogenously ; after 3 days, a thin pellicle developed at the surface ; growth in the line of puncture was very feeble, beaded or filiform. The liquid showed alkaline reaction and red litmus paper hung in the tube turned blue, indicating production of ammonia.

(*5*) *Glucose added to gelatin stab.* Surface growth occurred but no liquefaction.

(*6*) *Nutrient broth.* After 1 day at 28°C., homogenous clouding occurred accompanied by some sediment ; after 3 days, a yellow brown pellicle was produced at the surface ; after a week, the medium became rather clear, precipitating much sediment.

(*7*) *Milk.* After 2 days at 27°C., casein coagulated and soon afterward began to peptonize ; after 3 weeks had passed, testing of the medium gave fairly acid reaction.

(*8*) *Litmus milk.* After 1 day at 27°C., discoloration began to occur ; a thin pellicle was produced at the surface. After 2 days, the medium turned light yellowish brown.

(*9*) *Gelatin colonies.* After 2 days at 19°C., minute colonies appeared, causing liquefaction from the following day. Surface colonies : larger than buried ones ; yellowish brown to grayish yellow, usually round, entire edged, compact, but occasionally spreading faint net-like extensions around the central compact colony. The liquid surrounding the colonies was thinly clouded and sometimes accompanied with radial rays around the liquefied zone.

(PL. VI. 9. a. b. c.) Buried colonies : small, round or elliptical, compact, possessing no extensions. (PL. VI. 9. d.)

(*10*) *Agar colonies.* After 1 day at 27°C., tolerably large colonies developed, which were usually light yellow, sometimes grayish white. They showed tendency to vary with variation of temperature and other factors, assuming several different varieties of form. Surface colonies were usually amoeboid, possessing lobate or finger-like or fimbriate outgrowths at their margin (PL. VI. 2. 3. 4.), but sometimes roundish in outline, showing smooth or net-like folds or concentrically ringed surface. (PL. VI. 5. 6.) Observing such different forms, one might think that they belonged to different species. I have, however, ascertained, by comparative studies of cultures, employing agar media, steamed potato, etc., that all of them may be regarded as identical, i. e., as belonging to the same species.

Buried colonies are small, yellowish brown or grayish yellow, somewhat irregularly spindle-shaped or elliptical, occasionally possessing a few slender outgrowths at their periphery. (PL. VI. 8.) Bottom colonies are very thin, round, usually showing a light brown ; compact, origina. colony in the middle. (PL. VI. 7.)

(c) Physiology

(*1*) *Ammonia production.* This bacillus can produce ammonia gas from several culture media as S bacillus does, but its action is somewhat weaker than that of the latter.

Culture media	Turning blue of red litmus paper			Nessler's reaction
	after 1 day	after 2 days	after 3 days	
Uschinsky's solution	−	−	+	++
Chon's solution	−	−	−	?
Urea solution	+++	+++	+++	+++
Winogradski's solution	+	+	+	++
Egg-white	+	+	+	...
Peptone water	−	+	+	++

Note : Preparation of media, incubation temperature, method of detection, etc.

are the same as in the case of S bacillus.

As the result, protein of egg-white, peptone, ammonium sulphate in Winogradski's medium, urea, and asparagin in Uschinsky's solution were decomposed by the bacillus, producing ammonia.

(2) *Nitrite production.* Culture of nitrate peptone solution for 10 days at 27°C. was tested by Griess' reaction, exhibiting a feeble positive reaction, i. e., light pink color; while ordinary peptone solution, containing no nitrate, indicated negative reaction.

(3) *Temperature relation.* Growth of M bacillus at various temperatures is tabulated below;—

Temperature applied (C).	State of growth
0°	−
10°	−
14°	−
21°	+
25°	++
29°	++
35°	+++
37°	+++
40°	+++
44°	++
47°	++

According to the above table, growth of M bacillus does not occur below 14°C. is feeble at 21°C.; optimum temperature is between 35°C. and 40°C.; moreover the bacillus develops at over 47°C., so that it is to be assigned to the thermophiles.

(4) *Resistance to sunlight.* Result of exposure for one hour was not very different from that with the control (not exposed); exposure for over 3 hours caused considerable decrease in number of colonies; the bacilli almost entirely destroyed by 6 hours exposure.

(5) *Indol production.* No reaction was to be seen on testing for indol

by Salkowski's method with broth cultures 1, 2, and 3 weeks old respectively at 27°C.

(*6*) *SH_2 production.* In milk culture, lead-paper hung in tube began to brown at its lower end after 6 days at 72°C., and the degree of pigmentation gradually increased; in broth culture, it began to brown after 3 days.

(*7*) *Relation to oxygen.* In Buchner's anaerobic culture, a scanty growth occurred, but soon ceased its development. After 23 days had passed, air was introduced, when quick development followed and a thick layer was formed, showing that this organisms is strictly aerobic.

(*8*) *Resistance to acid and alkali.* Growth of M bacillus was examined by cultivating in various acid and alkaline bouillons, and the following results obtained :—

Note : Designations are the same as those for S bacillus.

Culture media		Growth	Pellicle
acid	40°	−	absent
〃	30°	−	〃
〃	20°	+	〃
〃	10°	++	slight
neutral	0°	+++	〃
alkaline	10°	+++	abundant
〃	20°	+++	〃
〃	30°	++	absent
〃	40°	++	〃
〃	50°	+	〃
〃	60°	−	〃

The optimum reaction in growth took place between neutral and 20° alkaline and the growth decreasing in vigor outside that range and finally ceasing altogether at 30° acid or 60° alkaline.

(*9*) *Enzyme.* Tests for several enzymes were made by the same methods as in the case of S bacillus, with the following results :—

Diastase	present
Invertase	〃

Lipase	〃
Oxidase	absent
Peroxidase	〃
Tyrosinase	present
Proteolytic enzymes	〃

(*10*) *Acid production.* Bouillons with cane-sugar or glycerine added gave clear acid reaction after 6 days at 27°C, but with lactose added no acid was produced.

(d) Identification of species.

M bacillus seems to be identical with Bacillus mesentericus (*Flügge*) *Lehm. et Neum.* No radical differences exist between them, as the comparison in the following table shows :—

	Bac. mesentericus	M bacillus
Size of organisms	0.8−2.4μ ×0.7−0.9μ	2.6−3.2μ ×0.5−0.6μ
Endospores	Almost round.	Elliptical, 2.4×1.1μ
Gelatin colonies	Surface clonies are small, round, white ; liquefaction occurs forming a gray clouded zone around the colony ; the central part of the colony is compact and grayish brown ; the periphery finely fimbriate. Buried colonies are grayish yellow, irregular shaped, possessing hair-like outgrowths at their periphery.	Surface colonies are round, entire edged, yellowish brown to grayish yellow ; at times, spreading net-like extension around the initial colonies. Buried colonies are round or elliptical, possessing no outgrowth.
Gelatin stab	Liquefaction occurs in crateriform within from 12 to 24 hours, then becomes infundibuliform to stratiform ; the liquid clouds with a floating grayish white pellicle at its surface.	No remarkable difference.
Agar colonies	Surface colonies are irregular, gray to yellowish, fine granular. Buried colonies are roundish, yellowish brown, entire or fimbriate edged.	Surface colonies are light yellowish brown to grayish white ; amoeboid or round shaped. Buried colonies are yellowish brown to grayish yellow, somewhat irregular spindle-shaped or elliptical, possessing a few slender outgrowths at their periphery.

Agar stroke	Growth yellowish brown, glistening, lobate edged; condensed water clouds, accompanied by a pellicle at the surface and some yellowish sediment.	Almost the same.
Broth	Clouding occurs with a floating pellicle at the surface.	The same.
Potato	Growth, at first, is grayish yellow, glistening, slimy; soon becomes yellowish gray, producing peculiar anastomosing folds.	Almost the same.
Indol	Produced slightly.	Absent.
SH_2	Produced abundantly.	The same.

Chapter III. COCOON FUNGI.

A. Historical Account.

It is well-known that cocoons are often spoiled by fungi when they have been stored for a long time. As such cocoons are not only difficult to reel but also yield inferior quality of fibre, they are very much disliked by silk-manufacturers.

Literature concerning cocoon fungi, is confined to the reports of *Nomura* and *Iwafuchi*.

H. Nomura (1897) previously identified 2 species, namely, Aspergillus glaucus and Aspergillus flavus from the infected cocoons, and showed that (1) the germs of these fungi are actually floating in the air of the silk-worm rearing room; (2) the fungi seem to obtain entrance into the larvae through the spiracles rather than through the mouth; (3) the cocoon fungus thus seems to begin in the body of the pupa and thence to extend gradually to the cocoon, where it finally makes its way to the outer surface; (4) the spores of these fungi do not lose their vitality on passing through the alimentary canal of the silkworm; (5) desiccation for eight hours at a temperature of 70°—

75°C. is not sufficient for killing the spores of the fuugi.

H. Iwafuchi, subsequently mentioned four kinds of cocoon fungi, i. e., Eurotium herbariorum, Aspergillus flavus, Penicillium glaucum, and Mucor mucedo. Of these the first is the most common, the third is next, the second is rather rare, and the fourth usually develops on putrefied cocoons.

B. Morphology, Culture, and Identification, of the Cocoon Fungi obtained in my Experiments.

The cocoon fungi which are to be described in the following pages were obtained from cocoons which had been in storage in the various departments, sericulture, filature, and spinning, of the Uyeda Silk Technical College, during 1921 and 1922.

(a) C. F. no. 1.

(=Aspergillus glaucus *Link*)

(1) Morphological Features

This fungus, a parasite on the cocoon, at first exhibits light yellowish green spots which become yellowish brown to dark brown when older. The young conidial herbage, developed on the pupa, is pale green to verdigris-coloured but darkens quickly to a dirty grayish green or grayish brown. The vegetative hyphae are branching and septate, consisting of raws of cells, 4 to 6μ wide. The conidiophores are the largest among the cocoon fungi, the length being 550—1260μ, commonly 900μ and the width 12—20μ, commonly 15μ. The globule, which is not sharply demarcated from the stalk, is spherical, measuring 48—70μ, commonly 57μ in diameter, but when artificially cultivated with Soya or gelatin media its size is considerably reduced, becomeing merely 18—37μ across. The sterigmata are thickly attached all over the globule, measuring 12—15μ long and 7—9μ wide, ovate or elongated ovate, bearing numerous conidia in chains. The conidia are large, prickly, globular or slightly elongated, thick walled, measuring 7—10μ in diameter.

Perithecia are produced with great readiness and in abundance, on the

fungous herbage, where they are to be seen macroscopically as yellowish granules. They measure 60—135μ, commonly 90μ in diameter, with a simple, delicate envelope made up of pseudoparenchymatous hyphae, enclosing numerous rounded oval asci. Matured asci measure about 18μ long by 13μ broad, containing usually 8 colorless, smooth, ellipsoidal spores, which exhibit a furrow around their side, measuring 5—8μ long by 4—5μ thick. (PL. X. 1—9.)

(2) Cultural Features

Cane-sugar added to agar stroke. After 1 day at 22.5°C., faint growth appeared ; after 2 days became colorless, opaque, 3mm wide ; after 3 days turned white, but bluish green in the middle part ; after 4 days the herbage, measuring 15mm wide, was somewhat higher and showed grayish color along the streak line, both sides of which were grayish blue, the margin white, and the upper region dark green (PL. IX. 1.) ; after 5 days the middle part of the herbage darkened and the upper part browned ; after 6 days the whole surface of the herbage became darker color.

Ordinary agar stroke. Growth was much inferior to that of the above culture, developing bluish green herbage, measuring 6mm wide after 6 days ; color of the herbage did not vary in the different parts.

Gelatin stroke. After 1 day at 19°C., a faint growth appeared along the streak line, forming afterwards white herbage ; 3 days later it became blue but no liquefaction occurred.

Potato. After 2 days at 25°C. a grayish white herbage developed, measuring 3mm in diameter ; after 3 days it attained a width of 8mm appearing light blue in the central part ; after 6 days the inner part turned grayish blue, while the margin remained white.

Bread. After 3 days at 25°C. a very thin grayish herbage developed, measuring 25mm in diameter ; after 6 days it spread over the whole surface of the medium, producing blue conidiophores sparsely ; after 8 days the middle part became yellowish, subsequently forming many orange yellow perithecia.

Miyoshi's Soya. (Cultivated in small Erlenmyer flask.) After 2 days at 25°C. small colonies developed on the surface ; after 3 days spread widely, occupying about 8/10 of the surface of the medium, and bearing pale green conidio-

phores (PL. IX. 7.) ; 2 weeks later the herbage became yellowish, bearing numerous perithecia.

Steamed rice. After 4 days conidiophores were produced very sparsely ; a month later became yellow, forming many perithecia.

Litmus milk. The color of litmus was gradually reduced but no defined colonies developed at the surface.

Uschinsky's solution. After 1 week, no growth was to be seen, but 2 months later small cotton-like masses appeared in the medium which turned somewhat brown ; after 3 months the mycelial masses as well as the medium were quite brown and some conidiophores were produced sparsely on the inside wall above the medium.

Winogradski's solution. Showed very scanty growth ; after 2 months faint mycelium developed on the inside of the tube, above the surface of the medium, and subsequently produced conidiophores sparsely on it ; afterward the aerial mycelium turned dark brown, but the medium was not discolored.

(3) Identification of species

C. F. no. 1. may be the same fungus as Aspergillus herbariorum *Fischer* (= Eurotium aspergillus glaucus *de Bary*) mentioned in Engler-Plantl's 'Natürliche Pflanzen Familien' and as Aspergillus glaucus *Link* described in Lafer's Techinical Mycology, although some differences are to be seen on comparison, as shown in the table below :—

	A. herbariorum	A. glaucus	C. F. no. 1.
Perithecium	Sulphur yellow, 75—90μ in dia.	At first light yellowish brown, later dirty brown, 100—200μ in dia.	At first light yellow. later light brown, 60—135μ average 90μ in dia.
Ascospore	8—10μ in dia., 5—7μ thick.	7—10μ in dia., 5—8μ thick.	5—8μ in dia., 4—5μ thick.
Conidiophore	1mm height.	1—3mm.	0.55—1.26mm.; average 0.9mm.
Globule	20—40μ in dia.	60μ .	48—70μ ; average 57μ .
Sterigmata	.....	14×7μ .	12—15μ ×7—9μ .
Conidia	9—15μ in dia.	7—30μ .	7—10μ .

We see, in the above table, that there exist no essential differences among them but only the slight variations of type, which are to be expected.

(b) C. F. no. 2.
(=Asp. glaucus *Link* var. α *Yendo.* n. var.)

(1) Morphological features.

The cocoon invaded by this fungus exhibits yellow or yellowish green spots on its surface and the pupa is covered with a beautiful yellow herbage. The hyphae are 4—6μ thick, containing fine yellow granules in every cell and branched repeatedly, showing many spirally coiled gnarls which finally develop into perithecia.

The perithecium is similar to C. F. no. 1, measuring 70—170μ, commonly 100μ in diameter. The ascospores are also the same as with the former. The conidiophores are at times produced sparsely on the thick yellow herbage, their length being 350—500μ and their width 9—15μ. The globule, which is not sharply demarcated from the stalk, is ovate or knob-like, measuring 12—35μ across, and thickly covered with simple sterigmata at its summit. The sterigmata are ovate or long elliptical, measuring 8—13μ long by 5—7μ wide. The conidia are produced in chains from the tips of the sterigmata; spherical, fine prickly, measuring 5—7μ across. (PL. X. 10—16.)

(2) Cultural features.

Cane-sugar added to agar stroke. After 2 days at 22.5°C. growth appeared as colorless opaque spots along the streak line; on the following day they became white measuring 2*mm.* across; after 4 days the colonies developed to form a continuous herbage, attaining a width of about 10*mm.* and the central part was somewhat raised and of a fresh yellow color; after 5 days transversal folds occurred in the herbage and its upper part browned, moreover the color, throughout, darkened day by day. (PL. IX. 3.)

Ordinary agar stroke. Growth is greatly inferior to that of the preceding culture; 4 days later, the herbage looked yellow and began to change to dirty yellow after 6 days, measuring 7*mm.* across.

Gelatin stroke. After 5 days at 19°C. a slight development occurred ; succeeding growth was also feeble, accompanied by no liquefaction.

Steamed potato. After 2 days at 25°C. fungus appeared as a small white patch ; on the following day increased in width up to 4*mm.* ; after 9 days measured 16*mm.* across, becoming yellow at the middle part which subsequently turned brown and protruded somewhat exhibiting radial folds ; the color of the medium beneath the herbage changed to dark brown.

Bread. Growth developed very thinly ; its periphery was not clearly bounded ; it appeared light yellow at the middle ; afterwards numerous perithecia and some conidiophores were produced on it.

Miyoshi's Soya. After 2 days at 25°C. a slight development occurred ; after 3 days it became a round herbage whose margin was white and middle fresh yellow containing numerous perithecia ; after 4 days great folds occurred bending up the margin here and there, and bluish conidiophores were produced sparsely on the surface ; afterwards the color of the herbage gradually faded to yellowish brown. (PL. IX. 8.)

Steamed rice. After 4 days bluish conidiophores were produced sparsely on the surface ; after 5 days perithecia began to form and 2 weeks later the perithecia alone remained, the conidiohores having entirely disappeared.

Litmus milk. No herbage formed at the surface of the medium, and no marked change occurred in color.

Uschinsky's solution. After 5 days, a little mycelium developed at the surface of the medium ; after 2 months colorless semitransparent mycelial masses were seen at the surface as well as at the bottom ; after 3 months color of the medium turned light yellow, and at the same time the mycelial masses became light yellowish brown ; afterward some perithecia were produced in the surface herbage but contained no spores.

Winogradski's solution. After 2 months, white cotton-like mycelia appeared in the medium and also thin mycelia around the inside wall of tube above the surface of the medium, the latter afterwards producing some conidiophores and perithecia ; moreover the medium turned dark purple and the hyphae brown.

(3) Identification of species.

Comparing the present species to C. F. no. 1, we learn that they are closely related in their morphological features, i. e., the perithecium and the ascospore are almost the same, the conidia also closely resemble each other in appearance although they vary a little in size. What differs in the two is that the present species is richer in producing perithecia, but poorer in producing conidiophores so that the two differ greatly in the coloration and appearance of their herbage.

Considering these characteristics, it may be preferable to consider the present fungus as a variety of ordinary Aspergillus glaucus; I consequently record it provisionally under the name of A. glaucus var. α.

(c) C. F no. 3.
(= Aspergillus glaucus *Link.*
var. β *Yendo*, n. var.)

(1) Morphological Features.

The fresh herbage of this fungus is a deep blue color, but when older it fades to dark grayish blue. This fungus usually produces conidiophores only, very seldom perithecia. The conidiophore is similar to that of C. F. no. 1, the globule being ovate or spherical, measuring 15—40μ commonly 27μ in diameter; the sterigmata 12—15μ long by 5—8μ wide; the conidia are spherical, finely prickled, measuring 6—9μ across; the perithecia and ascospore resemble greatly those of C. F. no. 1, so that it is almost impossible to distinguish one from the other. (PL. X. 17—19.)

(2) Cultural Features.

Agar stroke with cane sugar added. After 2 days at 22.5°C. faint growth appeared; after 3 days, developed as a colorless semitransparent layer, measuring 3*mm.* across; after 4 days, became white increasing in thickness; after 5 days the upper part of the herbage showed a light blue color. (PL. IX. 5.)

Ordinary agar stroke. After 1 day growth appeared as a white line

along the streak line and on the following day became blue ; after 5 days faded to grayish blue, attaining width of 8*mm*.

Gelatin stroke. After 1 day at 19°C. scanty growth occurred and 4 days later developed to a white herbage, which afterward turned blue. Liquefaction of the medium did not occur.

Steamed potato. After 2 days at 25°C. appeared as a white patch, measuring 3*mm*. across, subsequently the middle portion turned blue and then dark blue, forming wavy folds.

Bread. After 3 days, grown to a size of 10*mm*. across, raising aerial hyphae on the herbage, whose middle part appeared blue and the periphery yellow ; after 6 days attained to 23*mm*. in diameter, and subsequently the herbage became dark blue, the yellow margin disappearing. No perithecium was formed.

Miyoshi's Soya. After 2 days, developed a little, and on the succeeding day the herbage covered the greater part of the medium. The color of this herbage is deeper blue and its surface more rugose than that of C. F. no. 1. (PL. IX. 9.)

Steamed rice. Growth scanty ; after 3 days bluish conidiophores were produced very sparsely and 2 weeks later some yellow perithecia appeared.

Litmus milk. At first the litmus turned red, and afterward lost its color, and peptonization of casein occurred.

Uschinsky's solution. After 5 days some mycelii produced, bearing a few conidiophores, on the surface of the medium ; at the same time mycelial masses appeared at the bottom of tube ; after 3 months the mycelii changed to a brown color, the medium also turned a light brown.

Winogradski's solution. After 2 months scanty mycelium produced around the inside of tube above the medium, bearing grayish green conidiophores. Almost no growth was to be seen in the medium.

(3) Identification of species.

The present species agrees with C. F. no. 1 in several important morphological characteristics such as the shape and size of conidia, ascospores, etc.,

but it differs in such characteristics as, in general, producing merely conidiophores, perithecia being formed very rarely. These points being considered, it is designated with the name of Aspergillus var. β.

(d) C.F. no. 4.

(=Aspergillus flavus *Link*)

(1) Morphological features.

The cocoon invaded by this fungus exhibits light grayish green or brown spots at its surface; the pupa is covered with a brownish yellow or green herbage, which turns dark brown when older. The conidiophores which measure 340—610μ, average 480μ long by 5—8μ wide, carry a spherical globule which measures 18—31μ, commonly 25μ in diameter; its stalk is very characteristic and diagnostic since it possesses minute granules around its surface. The sterigmata are simple, slender, measuring 7—12μ long by 4—5μ wide, generally disposed radially though sometimes confined to the summit, and developing conidia from their tip. The conidia are arranged in chains, globular, measuring 4—6μ in diameter; their surface smooth when young but finely granular when older; soon separated by constriction into chaplets, which readily become dissociated. (PL. XI. 1—6.) No perithecium was observed.

(2) Cultural features.

Agar stroke with cane sugar added. After 1 day at 22.5°C. whitish growth appeared along the streak line; after 2 days attained to 5*mm.* in width; after 3 days spread over the whole surface, sending up white aerial hyphae at the middle line, both sides of which exhibited coarse, transversal, yellowish folds, the depressed furrows as well as the upper part of the slant being green; after 5 days the whole had become green. (PL. IX. 1.)

Ordinary agar stroke. After 2 days a brownish yellow herbage appeared and 6 days later turned darker, but never becoming green as did the above.

Gelatin stroke. After 1 day at 19°C. white growths appeared along the streak line; after 3 days the middle part became green, simultaneously with liquefaction of the medium.

Steamed potato. After 1 day at 25°C. a white patch developed ; after 2 days it attained to a size of 15*mm.* in diameter, showing yellowish green color at the middle and white floccose hyphae at the periphery ; after 6 days was 23*mm.* across. (PL. XI. 1.)

Bread. After 3 days developed to a size of 19*mm.* with many white aerial hyphae and yellowish conidiophores in the middle ; afterwards the herbage increased more and more, a green shade appearing in the middle which finally became dark green.

Miyoshi's Soya. After 1 day small white colonies appeared on the surface of the medium ; after 2 days they became large enough to fuse together, covering the whole surface ; after 3 days yellow conidia were produced abundantly and large irregular folds occurred in the herbage ; after a week the whole turned dirty yellow. (PL. IX. 6.)

Steamed rice. After 2 days green conidiophores were produced abundantly; after 3 days the hyphae developed to 2*mm.* below the surface.

Litmus milk. The herbage developed on the surface of the medium, which at first turned red and later light brown with rapid peptonization.

Uschinsky's solution. After 5 days, numerous small colonies developed around the whole inside of tube ; after 3 months some conidiophores appeared on the surface of tube above the medium, but no discoloration of the medium occurred.

Winogradski's solution. After 1 week, numerous white star-like colonies developed in the medium around the inside of tube ; afterwards they assumed a light brown color, and yellowish green conidiophores were produced sparsely around the wall of tube above the medium.

(3) Identification of species.

The present species agrees, in the important features, with Aspergillus flavus *Link*, so that we must recognize it as synonymous. Some differences are tabulated below : —

	A. flavus	C. F. no. 4
Conidiophore	500—700μ.	340—610μ, ave. 480μ.
Globule	30— 40μ in dia.	18— 31μ
Sterigmata	20— 60μ long.	7—12μ long, 4—5μwide.
Conidia	4 — 8μ in dia.	4—6μ.
Perithecium	not present.	ditto.

(e) C. F. no. 5

(= Aspergillus fumigatus *Fres.*)

(1) Morphological features.

The present fungus attacks the cocoon, producing dark green to grayish green spots on its surface and also similar colored herbage on the pupa, in which occasionally initial young mycelii are observable in small masses, emerging from the spiracles. (PL. VIII. 6.) The conidiophores are dwarf, measuring 80—560μ long by 5—10μ thick. The globule is club-shaped, 12—18μ in diameter, possessing many simple, slender, upright sterigmata (8—10μ long by 2—3μ thick) at its summit. The conidia are very small, measuring 2—3μ across, with minute granules over the whole surface. (PL. XI. 7—9.) No perithecium was observed.

(2) Identification of species.

This fungus agrees with Aspergillus fumigatus *Fresenius* in several essential features as may be seen in the following table: —

	A. Fumigatus	C. F. no. 5
Conidiophore	100—300μ long.	80—560μ, ave. 375μ.
Globule	10—20μ in dia.	12—18μ.
Sterigmata	6—15μ long.	8—10μ long, 2—3μ wide.
Conidia	2—3μ in dia.	2—3μ.
Perithecium	not formed.	the same.

(f) C. F. no. 6.

(= Aspergillus albus *Wilhelm*)

(1) Morphological features.

Cocoon invaded by this fungus, at first, do not show any conspicuous discoloration but later turn cream-color or light brown. The conidiophores are 300—730μ, commonly 500μ long by 6—9μ thick. The globule is sharply defined, spherical, thick-walled, measuring 30—40μ in diameter, bearing branched sterigmata radially. The primary sterigmata are large, wedge-shaped, measuring 33—38μ by 5—7μ, each carrying 3 or 4 ornamental secondaries which are slender, cylindrical, measuring 8—10μ by 2.0—2.5μ. The conidia are small, globular, smooth, 3μ across. (PL. XI. 10—12.)

(2) Cultural features.

Steamed potato. After 3 days at 25°C. a herbage developed, measuring 8*mm.* in dia., raising cotton-like hyphae on its surface; later gradually increased in size but was not discolored for a long time. (PL. XI. 10.)

Bread. After 3 days developed to a size of 1 *cm.* in diameter, showing white floccose hyphae at the middle; 2 weeks later the herbage turned cream-color.

(3) Identification of species.

There are some kinds of white fungi possessing branched sterigmata in the genus of Aspergillus, i. e., A. albus *Wilhelm*, A. candidus I *Wehmer*, A. Okazakii *Yagi*, etc.

The descriptions of the first one, given by *Wilhelm* in 1877, are in rather confused state so that precise comparison is difficult.

The second one occurs chiefly on old, decayed vegetables of various kinds, as well as on putrescent urine, and old cheese; its herbage is white at first, turning creamy in old cultures, and even brown in those on wort gelatin; it exhibits two formes of conidiophores, one with spherical globule and branched sterigmata, whilst the other is much simpler and smaller, the sterigmata

being unbranched ; the conidia are mostly ellipsoidal, smooth or coverd with fine dots, and 2.5—4μ in diameter.

The two species just mentioned are probably identical but at present it is impossible to say with certainty that they are so.

The third one was isolated by *K. Okazaki* and given its nomenclature by *K. Yagi*. This species, however, shows no great differences, in comparison with the two above, so that it is doubtful whether they are really different species. The following table compares the morphological features of A. Okazakii and C. F. no. 6.

	A. Okazakii	C. F. no. 6
Conidiophore	200—500μ long, 8—12μ thick.	300—730μ, ave. 500μ × 6—9μ.
Globule	12—40μ in dia.	30—40μ,
Sterigmata	Primaries : 16—20μ×6—8μ. Secondaries : 8—14μ×3.6μ.	33—38μ × 5—7μ. 8—10μ × 2— 2.5μ.
Conidia	2.5—5.4μ in dia.	3μ.

We see from the above table that no conspicuous difference exists between them, but rather close alliance ; consequently the older name, A. albus, may be adopted for C. F. no. 6.

(g) C. F. no. 7.

(= Penicillium commune *Thom*)

(1) Morphological features.

The fresh herbage of this fungus is compact, villose, grayish green to dark gray in color. The hyphae are 2.5—3μ thick with minute granules on the surface. The conidiophores, verticillately branched at the tip, are 25—50μ high by 3—4μ thick. The conidia are globoid or oval, smooth, 3—4μ average 3.7μ in diameter, and adhere to each other in long chains. Perithecia were not produced. (PL. XI. 13—18.)

(2) Cultural features.

Gelatin stroke. After 1 week at 19°C. growth occupied about half the

area of the surface, its margin being grayish white and the inner part grayish blue ; later white hyphae were produced on the herbage and some hyphae intruded into the medium, no evidence of liquefaction, however, occurring.

Steamed potato. At first developed as a white patch which soon became compact herbage, the margin being white and the inner part bluish green. In older culture, some radial folds appeared in the herbage and a few small water drops on the surface. (PL. XI. 13.)

Bread. After 3 days at 25°C. produced a herbage, measuring 8*mm.* across ; its margin being white and the middle bluish green ; the herbage gradually increased in area.

(3) Identification of species.

The present fungus is compared briefly with Penicillium crustaceum *Linn.* as well as Penicillium glaucum *Bref.* in the following table : —

	P. crustaceum	P. glaucum	C. F. no. 7
Conidiophore	100—200μ.	200—400μ.	25—50μ,
Conidia	2—3μ in dia.	2.5μ.	3—4μ, ave. 3.7μ.
Perithecium	formed.	the same.	not formed.

As the size of conidia of P. glaucum varies in the reports of observers, measuring for instance, 2—3μ according to *Shröter*, 3μ according to *Wehmer*, 4μ according to *Lindau*, and 3.8—4.3μ according to *Stoll*, it cannot, therefore, be distinguished from C. F. no. 7 by the size of the conidia. The first two species, however, possess the characteristic of readily producing perithecia, while mine does not. *Thom* performed precise investigations on the genus Penicillium. According to his classification, C, F, no. 7 seems to be identical with P. commune *Thom*, since it forms neither coremia nor perithecia, its growth is not accompanied by rapid liquefaction of gelatin, and it produces green compact herbage with floccose surface hyphae.

(h) C. F. no. 8.

(= Penicillium brevicaule *Sac.*)

(1) Morphological features.

Cocoons invaded by this fungus exhibit yellowish brown coloration and the pupa is covered with grayish brown, floccose herbage. This fungus attacks not only cocoons but frisons and other waste silks causing serious damage by producing brown discoloration. The hyphae are septate, containing many fat-granules in every cell; the thickness varies, of course, according to parts of the mycelium; considerable differences may also occur according to the culture medium; hyphae developed at the surface of cocoons, for instance, being 4μ in thickness, those developed in nitrate pepton media 2μ, those developed on bread 3μ, those developed on starch paste 5.0—2.5— 1.2μ, those developed on agar media 4—5μ, those developed on steamed potato 2.3μ; in the last case, some of the hyphae are generally grouped together in parallel formation, making a bundle or rope. (PL. XII. 14.) The conidiophores are produced perpendicularly from the hyphae, in various shapes, i. e., monopodial; simply or irregularly branched, etc.

Their height is commonly 25—75μ but there are often very short ones (below 10μ). Their thickness is 3—5μ, about the same as that of the hyphae. The sterigmata are inverted-clubshaped or cylindrical, masuring 5—37μ in length by 3—6μ in thickness, large conidia dividing off from their tips in short or long chains. There are two forms of the conidia; one being spherical, 5—7μ in diameter, the other pear-shaped, 6—9μ × 5—6μ; the latter sometimes pointed at the apex and provided with a decided stem at the base; both forms thick walled, prickly in appearance and covered with coarse granules. (PL. XII. 1—13.) No perithecia nor sclerotia were observed.

(2) Cultural features.

Cane sugar added to agar stroke. After 1 day at 22,5°C. a faint growth appeared along the streak line; after 2 days, it developed, to a colorless semitransparent herbage, measuring 4_{mm}. across; after 3 days, changed to grayish brown, spreading widely over the slant; after 4 days, its upper portion became grayish brown, the middle portion grayish white and the lower portion dark and moist; after 5 days it became darker all over, producing some transverse irregular furrows at the slant. (PL. IX. 4.)

Ordinary agar stroke. After 2 days, a white herbage appeared along the streak line ; after 3 days turned grayish brown ; after 6 days the herbage covered almost the entire surface of the slant.

Gelatin stroke. After 3 days at 19°C. , a white line appeared along the streak line ; after 4 days, its inner part turned yellowish brown ; after 6 days, liquefaction of the medium occurred, and red litmus paper hung inside the tube turned blue in consequence of ammonia production.

Steamed potato. After 1 day at 25°C., a small herbage appeared ; after 2 days, developed to a size of 10*mm.* across, of a grayish white color and provided abundantly with aerial hyphae ; after 7 days, the older parts became grayish brown ; after 9 days, attained to 45*mm.* in diameter, exuding tiny drops of water on the herbage ; in the course of development the medium was gradually consumed until its substance was lost completely ; again, in older cultures, many snowwhite, globular mycelial masses varying in size were produced on the herbage. (PL. XIII. 1—4.)

Miyoshi's Soya. After 2 days, some fungus developed on the surface of the medium and some with in the medium, the latter being semitransparent and slimy ; it afterward developed well inside the flask, a part emerging above the medium.

Steamed rice. After 2 days, white hyphae developed on the surface of the grains ; after 4 days, the hyphae reached down to a depth of 1.5*cm.* from the upper surface, where the herbage had become grayish brown.

Litmus milk. A little growth appeared on the surface and the litmus first turned red, later the color faded, and the liquid was finally left a transparent, light brown.

Uschinsky's solution. Submerged hyphae developed within 2 weeks ; after 3 months, became a grayish white mycelial mass at bottom of tube.

Winogradski's solution. After 1 week, a thin grayish brown herbage developed around the inside of the tube as well as at the surface of the medium ; afterward white mycelial masses were produced in the medium.

Nitrate peptone solution. After 2 days at 30°C. , a small grayish white herbage formed on the surface ; after 4 days numerous small colonies appeared

like snow-flakes in the medium ; after ten days the surface herbage turned light brown. (PL. XIII. 6.)

Starch paste. After a week at 30°C., developed to a grade observable macroscopically, causing liquefaction of the medium ; after 2 weeks the upper part, 5*mm.* in depth, was liquefied and the floating herbage slightly browned ; after 3 weeks about 1*cm.* was liquefied and the uppermost part became transparent. The liquid was tested by Fehling's solution, the result showing clearly that invert sugar was produced in consequence of decomposition of starch by diastase secreted from this fungus.

(3) Identification of species.

Penicillium brevicaule, first mentioned by *Saccardo*, has dwarf irregularly branched conidiophores whose sterigmata are $16\mu \times 3.5\mu$, and the conidia spherical, 6.5μ in diameter, or pear-shaped, $10 \times 6\mu$, so that no conspicuous morphological difference is to be seen between my C. F. no. 8 and his species. Moreover, it is common to both that rapid liquefaction of gelatin is produced as well as the emission of much ammonia gas from several media.

According to *Thom*, there are two different varieties besides the ordinary P. brevicaule, i. e.,(1) var. album whose herbage is white or cream, spores rough ; (2) var. glabrum whose herbage is white or cream, spores smooth. In my researches, however, such varieties have not been observed.

Remarks ; In the course of my experiments. I have occasionally found several other fungi besides those mentioned in the foregoing pages, for example, Mucor, Macrosporium, and Cladosporium, but I omit the description of these fungi as they are rarely found in cocoons.

C. Resistant power of Fungous Spores.

(a) Resistance to heating.

Spores of the cocoon fungi, as a rule, have strong resistant power to heat, so that they survive the temperature of 70°—80°C. continued for 8 hours or more during the cocoon drying process, and can also develop after being immersed for some time in the boiling water as well as in the hot reeling

water. At what degree of heat, then, are the spores killed?

In order to make sure of this, I have experimented on their power to resist heat both in the dry state and in the presence of water, employing the spores of A. glaucus, A. flavus, P. brevicaule, and P. commune.

(1) Resistance to moist heat.

In this experiment, 5*cc.* of Miyoshi's Soya was put in each tube and sterilized by steam heat; then the medium was inoculated with the spores, kept for one hour at the required temperature, and incubated at 25°C. to discover whether or not growth would take place.

	A. glaucus	A. flavus	P. brevicaule	P. commune
50°	+	+	+	+
55°	+	+	−	+
60°	−	+	−	−
63°	−	+	−	−
65°	−	−	−	−
68°	−	−	−	−
70°	−	−	−	−

As the above table shows, A. flavus made the strongest resistance, surviving up to 63°C.; next were A. glaucus and P. commune, up to 55°C.; lastly, P. brevicaule, up to 50°C. It is evident that the spores could resist higher degrees of heat if the heat were applied during a shorter time than one hour.

(2) Resistance to dry heat.

Some spores were put at the bottom of sterilized Petri dishes, and heated for one hour at the required degree of temperature, then Miyoshi's Soya was poured into them and they were incubated at 25°C.

The results are tabulated as follows:—

	A. glaucus	A. flavus	P. brevicaule	P. commune
60°	+	+	+	+
70°	+	+	+	+

80°	+	+	+	+
85°	+	+	−	+
90°	+	+	−	+
95°	−	+	−	−
97°	−	−	−	−

That is, A. flavus persisted up to 95°C., A. glaucus and P. commune up to 90°C., and P. brevicaule up to 80°C., all showing stronger resisting power than in the experiments with moist heat.

(b) Resistance to anaerobic condition.

Some experiments were made, adopting Buchner's anaerobic culture method, with agar slant to which pupa decoction was added. Having been cultivated at ordinary temperature in the early part of July, 1922, the control showed development within 2 or 3 days, while in the anaerobic culture there was no development. After 3 months, the plugs of the anaerobic tubes were removed to admit air and observation made to see whether or not the fungi were still alive.

Fungi	Results
A. glaucus	killed
A. flavus	//
P. brevicaule	living
P. commune	//
A. glaucus var. α	killed
A. glaucus var. β	living
A. albus	//

(c) Resistance to sunlight.

Agar slant to which pupa decoction was added was inoculated with spores, and immediately exposed to direct sunlight (Oct. 3. 1922); after a certain time, it was incubated at 22.5°C. for development. The results showed, as seen in the following table, that the 3 species alike resist up to 6 hours or more.

	A. glaucus	A. flavus	P. brevicaule
1 hour	+	+	+
2 hours	+	+	+
3 〃	+	+	+
4 〃	+	+	+
5 〃	+	+	+
6 〃	+	+	+

(d) Enzyme test.

It may be supposed that fungi attacking cocoons or pupae secrete certain enzymes, which act on them in different ways. In order to make sure of this, the following experiments were performed.

With the aim of collecting enzyme solutions, the fungi were cultivated in Erlenmeyer flasks filled with Miyoshi's Soya, subsequently well developed herbages were taken out, washed immediately and repeatedly with clean water to remove the soaked Soya, then crushed into minute particles in a mortar, mixed with quartz sand, and a uniform amount of water or glycerin, and left for some hours; finally, the solution was filtered with filter-paper and the filtrates were used for the tests. Chloroform was also added to each tube to prevent putrefaction. The incubation temperature was 37°C. in all cases.

(*I*) *Diastase.* 1% starch solution was made and boiled; 10*cc.* of this solution was poured into each tube and 2.5*cc.* of the enzyme solution added. For detection of diastase, iodine and potassium iodide solution was used, to ascertain roughly the quantity of starch remaining after a definite length of time, and Fehling's solution was also used as test for invert sugar which is produced from starch by the action of diastase.

Fungi	Results
A. glaucus	+
A. glaucus var. α	++
A. glaucus var. β	++

A. flavus	++
P. brevicaule	++

Designation: + diastase present,
++ tolerable amount,
+++ abundant.

(2) *Lipase.* A small amount of oil of pupa was mixed in distilled water in each tube and an emulsion made by shaking; a few drops of litmus tincture and sodium carbonate solution were then added until a slight alkaline reaction appeared; and finally a fixed amount of the enzyme solution was poured in.

Observation was made on the succeeding days to note change to red of the litmus, which showed the production of fatty acid from oil by the action of lipase.

Fungi	Results
A. glaucus	+
A. glaucus var. α	++
A. glaucus var. β	++
A. flavus	+
P. brevicaule	+

(3) *Pepsin.* This was detected by observing, in slightly acid enzyme solution, conditions of swelling, decoloration and liquefaction of coagulated egg-white or fibrin which had been stained with carmine.

Fungi	Results
A. glaucus	−
A. glaucus var. α	−
A. glaucus var. β	−
A. flavus	−
P. brevicaule	+ ?

(4) *Trypsin* This enzyme was detected by observing, in slightly alkaline solution, effect on coagulated egg-white or fibrin stained with eosin.

Fungi	Results
A. glaucus	+
A. glaucus var. α	++
A. glaucus var. β	+
A. flavus	+
P. brevicaule	+++

Thus, trypsin was found in all the fungi, most abundantly in P. brevicaule, where the coagulated egg-white and fibrin were rapidly decolorized inflated and finally dissolved into a milky substance.

(5) *Tyrosinase.* 5*cc.* of saturated solution of tyrosin was poured into each tube and 2.5*cc.* of the enzyme solution added to it. Positive reaction was recognized if the solution become brown within a few days.

Fungi	Results
A. glaucus	−
A. glaucus var. α	+
A. glaucus var. β	++
A. flavus	−
P. brevicaule	+

D. Cocoon Fungi in Relation to Discoloration of Silk-Fibres.

Cocoons invaded by fungi at first turn various shades of color according to the different kind of parasites, but, in general, they become brown or dark brown when order. The most common cocoon fungi, as already mentioned, are A. glaucus, A. flavus, and P. brevicaule. As the last one among them seems to be the most harmful, I used that one chiefly in making my investigations.

Experiment I.

In the first place, with the aim of showing that discoloration of cocoons

is caused by parasitic fungi, pupae were removed by cutting the side of cocoons, and oily substances adhering to the silk layer were extracted by the Soxlet's apparatus, then the cocoons were put one in each tube, and sterilized by the intermittent steaming method. The cocoons were inoculated with spores of P. brevicaule and incubated at 30°C.

The result was that all the cocoons inoculated began to discolor within 2 days and the pigmentation became deeper with lapse of time, until it finally remained a clear brown color (PL. III. C. 4.)

From this result, we learn that, as with bacteria, brown discoloration can be caused by such fungi alone, even when the cocoons contain neither pupae nor oily substances.

Experiment II.

To observe wherther P. brevicaule can produce the brown discoloration of frisons, the present experiment was tried. Some of clear, white frisons produced in the filature department of the Uyeda Silk Technical College, being soaked with water were put in Petri dishes and sterilized by the intermittent steaming process. They were then inoculated with spores of P. brevicaule and left standing at the temperature of the room in June.

As the result, the region around the inoculated point began to turn yellowish brown within 5 days and afterward while enlarging its boundary became more and more deeply discolored, until it exhibited finally the same appearance as the so-called 'oily burn'. (PL. XIII. 7.)

In the above two experiments, we have learned the fact that silk-fibres are turned brown by P. brevicaule. We must now consider from what action the discoloration is derived, and the following experiments concern this question. As already mentioned, P. brevicaule, secreting trypsin, quickly dissolves such protein substances as egg-white and fibrin, liquefies gelatin and sets free much ammonia gas. From these facts it is evident that this fungus posseses strong proteolytic enzymes, but, in order to make still more sure, further experiments, of a different nature, were conducted employing sericin, fibroin, egg-white and bean-curd as the culture media.

Culture with sericin. The fungus developed very rapidly, being incubated at 27°C.; 10 days later the medium began to turn light brown beneath the fungous herbage, increasing in discoloration day by day. (PL. II. B. 4.)

Culture with fibroin. The fungi developed a little after 2 days at 27°C.; the medium was discolored very slightly after 2 weeks.

Culture with coagulated egg-white. After 2 days at 30°C., white hyphae were produced, with projecting conidiophores; the medium became transparent and brown beneath the fungous herbage and gradually liquefied; the liquefied region attained to 5*mm.* in depth from the surface after ten days and to one centimeter after 2 weeks. (PL. II. C. 4; PL. XII. 5.)

Culture with bean-curd. After 2 days at 30°C., white hyphae developed, forming gradually a thick herbage; the medium liquefied by and by, the liquid being a deep brown color.

As we have seen in these experiments, protein substances as sericin, fibroin, egg-white and bean-curd, suffer brown discoloration when acted on by the fungus. Such discoloration may be produced, as already mentioned in the case of the bacteria, through oxidation of tyrosin by tyrosinase, the tyrosin being derived from protein substances and the tyrosinase secreted in this case from the fungus. With the intention of demonstrating this clearly, the following experiments were made.

Culture with tyrosin added to agar stroke. After 5 days at 22.5°C. some hyphae developed along the streak line; after 1 week the herbage became light brown and at the same time the medium began to turn brown back of the herbage; after 1 month the whole was dark brown. (PL. IX. 10.)

Culture with tyrosin added to bouillon. After 2 days at 25°C., some hyphae were produced floating on the surface, soon forming a thick herbage; the bouillon became increasingly brown from 1 week later.

Thus, we learn clearly that tyrosin in culture media is turned brown by the action of the fungus, in other words, by the action of tyrosinase secreted by the fungus.

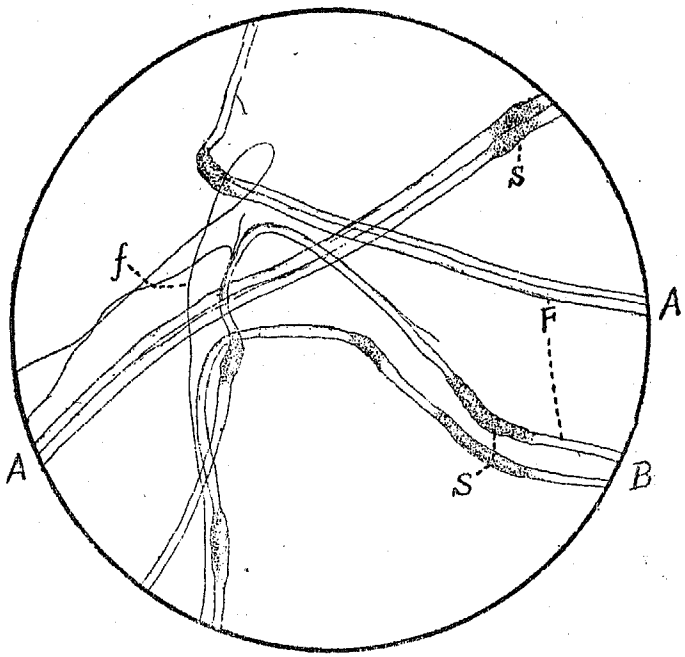
E. Character of the Discolored Silk-Fibres.

(a) Microscopical observation.

A small quantity of silk-fibre was cut from a discolored cocoon invaded by P. brevicaule and observed under the microscope, after application of a dilute eosin solution. I ascertained that the bave separated into two brins in consequence of losing the sericin cortex, and then into fibrils, the remaining sericin being well tinged with eosin but the fibroin cove, also fibrils, hardly stained.

Fig. 1. Microscopical view of silk-fibres injured by P. brevicaule.

A. Bave. S. Remaining sericin.
B. Brins. F. Naked fibroin.
f. Fibrils.



(b) Tenacity and elasticity of the discolored silk-fibres.

Silk-fibres invaded by the parasitic fungi not only discolor but show decided tendency to loss of strength. Some experiments with regard to these points are here described. At first, small silk skeins of 14 denier weight were put in Petri dishes, with a small amount of dilute pupa decoction and sterilized by the intermittent steaming process. Then the spores of several cocoon fungi were introduced into each dish and they were left standing during 52 days (from July 23rd to Sept. 13th, 1921). The strength of the fibres was then tested with the serimeter.

	Tenacity (mg)	Percentage of tenacity to control	Elasticity (mm)	Percentage o elasticity tof control
(1) Control	44.25	100	164.0	100
(2) P. brevicaule	30.45	68.8	195.3	119.1
(3) ditto	29.45	66.5	172.7	105.3
Average of (2) (3)	29.95	67.7	184.0	112.2
(4) A. glaucus	37.85	85.3	148.0	90.0
(5) A. g. var. α	40.35	91.1	151.0	92.0
(6) A. g. var. β	31.30	70.7	133.9	81.6
(7) A. flavus	34.05	76.9	130.4	80.0
(8) A. albus	40.10	90.4	168.0	102.4
(9) P. commune	41.95	94.8	186.8	113.9

Note: 1) In each division the record is the average of tests repeated 20 times.
2) To the control the pupa decoction was added, but it was not inoculated with spores.
3) In order to equalize the water content of the silk fibres, they were dried to some extent before applying the serimeter.

From the above table, the following deductions may be made:

(1) As regards tenacity, the control is the strongest and the divisions of P. brevicaule the most feeble.

(2) The average of tenacity in the two divisions (2)(3) of P. brevicaule is 29.95 mg. i. e., 67.7 % of that of the control.

(3) The elasticity varies with the divisions, showing no constant tendency, the divisions of P. brevicaule, for instance, exhibiting rather greater elasticity than the control, for which the reason is not clear.

F. Fungi Commonly Found in the Air of the Silkworm Rearing-Room.

With the aim of discovering the source of the fungi which are parasitic on cocoons and other silk substances, the air of the silkworm rearing-room was tested microbiologically in this way :— 4 Petri dishes containing acid agar medium were used each day ; the duration of exposure, the same each time, was 5 minutes, immediately after noon ; after the exposure the dishes were incubated at 25°C. for development of germs.

The experiments continued from the 4th to 13th of June, 1923, that is from the beginning of the fifth period until the cocoons were formed, The results tabulated are as follows :—

Date	No. of Petri dish	Kind and number of cocoon fungi		No. of other fungi	No. of bacterial colonies
4	1	P. brevicaule	2	5	9
		P. commune	3		
	2	P. brevicaule	2	2	5
		A. flavus	2		
		P. commune	2		
	3	P. brevicaule	2	0	6
		P. commune	4		
	4	A. flavus	1	2	13
		P. brevicaule	2		
		P. commune	3		
5	5	.....	0	0	0
	6	.....	0	0	1
	7	Mucor mucedo	1	0	1
	8	P. commune	1	0	2
6	9	P. commune	1	0	1
	10	.....	0	1	3
	11	.....	0	0	1
	12	(dish was broken)	...	...	...
7	13	P. commune	1	1	5
	14	.....	0	1	3

	15	P. commune	1	1	4
	16	.....	0	1	5
8	17	.....	0	0	1
	18	Macrosporium	1	0	4
	19	Macrosporium	1	0	2
	20	.....	0	1	4
9	21	P. brevicaule	7	3	4
	22	P. brevicaule	3	1	9
		A. glaucus	1		
		Mucor	1		
	23	P. brevicaule	5	2	14
		A. flavus	1		
		P. commune	4		
	24	P. brevicaule	8	0	10
10	25	.....	0	1	1
	26	.....	0	0	1
	27	.....	0	3	10
	28	.....	0	0	1
11	29	Cladosporium	11	2	19
	30	Cladosporium	8	3	27
		P. brevicaule	2		
		Macrosporium	1		
	31	Cladosporium	8	4	21
		Mocrosporium	1		
	32	Cladosporium	4	2	19
12	33	.....	0	1	3
	34	.....	0	0	6
	35	Cladosporium	1	0	1
	36	.....	0	2	3
13	37	.....	0	0	11
	38	P. brevicaule	1	0	16
	39	A. flavus	1	1	9
		Cladosporium	2		
	40	P. brevicaule	2	1	21

As shown in the above table, the cocoon fungi found in my present experiments are P. brevicaule, P. commune, A. flavus, A. glaucus, Mucor

mucedo, Cladosporium, Macrosporium, and of them the predominating one is P. brevicaule, of which 36 colonies were obtained in the 39 dishes.

G. Growth Temperature of the Cocoon Fungi.

According to the investigation hitherto made, it is recognized that many species of fungi flourish best at high temperatures (about 35°—40°C.), e. g., A. flavus and A. fumigatus.

On the other hand, A. glaucus, A. albus, etc., exhibit a preference rather low temperatures. I will state here, what I have observed in experimenting on the growth temperature of P. brevicaule. The method of this experiment was as follows :— A drop of bouillon was put on a cover-glass, with some spores added to it, placed in a Petri dish and some moist cotton laid near the cover-glass in order to prevent drying of the bouillon drop, then incubated at the required temperature. When 7 hours had passed, microscopical observation was made to determine presence or absence of germination, the number of germinating spores, and the length of germination tube.

The results are as follows :—

Temperature applied (C)	15°	20°	27°	30°	34°	37°	40°
No. of germinating spore (in %.)	0	5	18	42	18	12	0
No. of spore, with germination tube less than 10μ in length.	0	5	4	13	6	12	0
less than 20μ.	0	0	6	16	8	0	0
// 30μ.	0	0	5	9	4	0	0
// 40μ.	0	0	2	3	0	0	0
// 50μ.	0	0	1	1	1	0	0
Ave. length (μ) of germination tube.	0	4.2	17.7	16.1	11.4	8.1	0
Max. length of germination tube.	0	8.0	48.0	49.0	25.0	9.0	0

From the above table, we learn that, (1) 30°C. is the optimum temperature for growth, showing 42% of germinating spores within 7 hours; the next is 27°C. and 34°C., both showing 18% of germination within the

same period ; and 37°C. showed 12% of germination ; while a low temperature of 20°C. showed only 5%, and a high temperature of 40°C. showed no germination. (2) The length of germination tube is greatest at 30°C. and 27°C. and at either lower or higher temperature the length of germination tube decreases.

The preceeding experiment determined the extent of germination at the end of 7 hours. Another experiment was made to determine the extent of germination of the spores at the optimum temperature (30°C.) during various periods of time. (See Fig. 2.)

Duration of time.	State of germination.
After 4 hours	Some began to germinate.
After 5 hours	13% of spores had germinated; max. length of germinating tube being 12μ.
After 7 hours	42% of spores had germinated; max. length of germinating tube being 49μ.
After 15 hours	The greater part of spores had germinated; germination tubes developed into long hyphae often branching once or twice, provided with some septa; the longest hyphe measured 450μ.

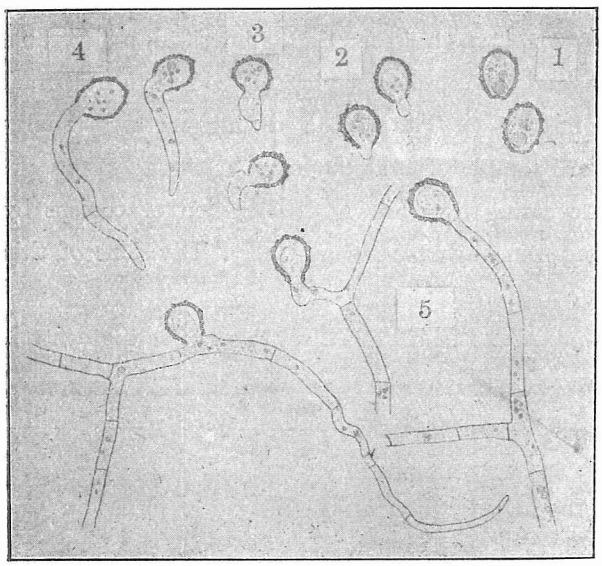


Fig. 2 : Progressive observation of the germination of spore of P. brevicaule.
1. spores before germination.
2. After 4 hours at 30°C.
3. After 5 hours.
4. After 7 hours.
5. After 15 hours.

Chapter IV. Methods of Preventing the Rusty-Brown Discoloration of Silk-Fibres.

In order te prevent the brown discoloration of silk-fibres caused by the microorganisms, it is evident that one thing at least is necessary, that is, the elimination of parasites.

It is, however, almost impossible to guard the silk from contact with them, because spores of the fungi as well as the bacteria float in the air everywhere in abundance. Our only choice is to use all precautions to suppress as thoroughly as possible the development or reproduction of the microorganisms which ever adhere to silk-fibres. The main expedients employed are dryiug, cooling, immersing water, and the application of chemicals.

A. Drying.

Microorganisms, as a rule, require moisture for their development, so that their growth is hindered in proportion as the quantity of moisture decreases. Drying is a preventative method in which a condition is provided, unfavorable for the development of germs, and the common drying method is to evaporate the moisture by means of heating. The cocoon drying method usually practised at present is just an example of heat drying.

I have succeeded in preventing the brown discoloration of cocoons by means of desiccators containing some hygroscopic acid clay. Divisions of this experiment are as follows :

Div. a. Moist cocoons were placed in a Petri dish, to serve as the control.

Div. b. Moist cocoons were placed in the desiccator.

Div. c. Moist cocoons, inoculated with S bacillus, were placed in the desiccator.

Div. d. Moist cocoons inoculated with M bacillus were placed in the desiccator.

Note : 1) Volume of the desiccator is 100*cc*.
2) Quantity of acid clay employed is 100*gr*. in each desiccator.
3) Outside layers of the cocoon exclusively were used for this experiment, in order to eliminate the effect of the pupa.
4) 10 cocoons were used for each division.
5) Temperature during the experiment was 30°C.

The following are the results : —

Div. a. After 1 day, a light brown discoloation occurred ; subsequently the pigmentation was intensified and numerous bacteria were found around the fibre.

Div. b. No discoloration occurred.

Div. c. The same.

Div. d. The same.

Judging from these results, we conclude that, on the one hand, moist cocoons as in division (a) become discolored by the action of the bacteria which adhere spontaneously to them and are propagated in the suitable moist condition ; on the other hand, however, cocoons dried as in divisions (b, c, d) exhibit no discoloration in spite of having been inoculated with the bacteria.

B. Cooling.

This method for preventing injury by the microorganisms, keeps the materials at such a low temperature that the parasites are unable to develop.

As already mentioned, S bacillus is thoroughly checked in its growth at the low temperature of 10°C. ; M bacillus, below 14°C. ; and P. brevicaule, below 15°C. Moreover, even A. glaucus. which is known as the one among the common fungi which endures the lowest temperature, ceases development when the temperature below 7°C. Therefore, we can prevent the discoloration of silk-fibres due to those microorganisms by keeping them at a low temperature, that is below 7°C. or 10°C. Divisions (4) and (5) of the experiment, concerning the discoloration of boiled cocoons, described in the chapter II, section B, may be consulted here.

C. Immersion in Water.

The silk-discoloring bacteria and the cocoon fungi, as already shown, are of necessity aerobic, exhibiting no growth in the anaerobic culture. The preventative method now under consideration is intended to suppress development of these aerobic microorganisms by immersion in water containing scanty oxygen, beside being at comparatively low temperature.

Division (2) in my experiment, described in chapter II—B, may serve as an example of this method.

D. Application of Chemicals.

Recently Chigasaki, Watanabe and Shimura (1922—'23) made attempts to prevent the putrefying of silk-reeling water as well as of boiled cocoons left in intervals of reeling, by applying such antiseptics as chloride of lime, carbolic acid, formalin, corrosive sublimate, Lugol's iodine solution, salicylic acid, potassium bichromate, hydrochloric acid, sulphuric acid, phosphoric acid, nitric acid, boric acid, formic acid, acetic acid, oxalic acid, hydrocyanic acid, citric acid, tartaric acid, chloride of amine, etc.

I proposed to disinfect cocoons, frisons etc. by means of fumigation, using various poisonous gases.

(a) Experiments on P. brevicaule.

Agar slants inoculated with spores of this fungus were introduced into a wide mouthed bottle of 1000*cc.* volume, and subjected to the action of a certain quantity of poisonous gases such as CS_2, chloroform, formalin, hydrocyanic acid gas, naphthalin, creosote, ether, and *Kiyonorin*,* during a certain time, then the slants were transferred to an incubator kept at 30°C. Results showed that 2 of these chemicals, i. e., CS_2 and chloroform, were more effective than the others ; I will therefore describe here the data of the experiments with these two chemicals only.

*A kind of antiseptic for preventing moulds, containing much ammonia as a constituent.

1) Fumigation with carbon bisulphide.

Quantity applied (*cc*)	Temperature during fumigation (C)	Duration of fumigation (*hours*)	Development of spore
0.08	18°	24	+
0.16	22°	24	+
0.16	25°	24	+
0.16	22°	48	+
0.16	27°	24	−

2) Fumigation with chloroform.

Quantity applied (*cc*)	Temperature during fumigation (C)	Duration of fumigation (*hours*)	Development of spore
0.3	18°	24	+
0.3	21°	24	+
0.5	25°	24	+
0.5	22°	48	+
0.7	27°	24	−

According to these tables, CS_2 is effective when applied at a strength of 0.16*cc.* and chloroform of 0.7*cc.*, for 100*cc.* of air, during 24 hours at 27°C. (nearly 80°F.). If we substitute amounts per volume of 1 cubic meter 160*cc.* of CS_2 and 700*cc.* of chloroform are required.

(b) Experiments on the bacteria S and M.

The bacteria were smeared on a sterilized cover-glass and put into a tube, then fumigated for 48 hours in a cylindrical bottle, 1000*cc.* in volume, containing a certain amount of CS_2. After this, the tube was taken out, a certain amount of bouillon added, and the tube incubated.

Quantity applied (*cc.*)	Temperature during fumigation (C)	Duration of fumigation (*hours*)	Development of	
			S bacillus	M bacillus
0.16	25°	48	+	+

0.20	25°	48	+	+
0.25	〃	〃	+	+
0.30	〃	〃	+	+
0.35	〃	〃	+	+
0.40	〃	〃	+	+

As the above table shows, neither bacillus is entirely destroyed even when fumigated with 0.4_{cc} of CS_2 for 1000_{cc} of air at 25°C. (i. e., over-saturated condition) for 48 hours.

The condition of this experiment, however, was unfavourable for making observation in consequence of bouillon having been used.

I therefore repeated the experiments, using agar slant. The bacteria, S and M in turn were inoculated into agar slants and closed up in cylindrical bottles of 1000_{cc} volume, to lie fumigated by the various doses of CS_2, at 25°C. during 48 hours; then the tubes were taken out and incubated. Observation was made during the fumigation as well as the incubation.

(1) S bacillus.

Quantity applied (*cc*)	State during fumigation	State after a week, of incubation
0.16	2 small colonies appeared along the streak line; condensed water some-what clouded.	The 2 colonies widely spread, cond. w. conspicuosly became turbid.
0.20	No growth on the slant; cond. w. a little clouded.	A few colonies appeared on the slant; cond. w. clouded.
0.25	The same.	The same.
0.30	〃	〃
0·35	〃	〃
0.40	〃	〃

(2) M bacillus.

Quantity applied (*cc*)	State during fumigation	State after a week, of incubation
0.16	A thick colony developed along the streak line; condensed water	Greatly developed.

	very clouded.	
0.20	Beaded colonies appeared along the streak line; cond. w. clouded.	The colonies widely spread; cond. w. conspicuously clouded.
0.25	No growth on the slant; cond. w. slightly clouded.	A thin colony developed; cond. w. somewhat clouded.
0.30	The same.	The same.
0.35	〃	〃
0.40	〃	〃

The above tables show that, (1) no conspicuous growth occurs during fumigation, when CS_2 was applied at a strength of over $0.2_{cc.}$ for S bacillus and over $0.25_{cc.}$ for M bacillus; (2) both S and M bacilli develop more or less in condensed water in spite of the presence of 0.4_{cc} of CS_2, that is in over-saturated air; (3) bacterial colonies developed on the slant after being fumigated, although no growth was seen during fumigation.

As above demonstrated, these bacteria are not totally destroyed by fumigation with CS_2, but there may be some hope of using this chemical to advantage in practice for preventing, to some extent, the deterioration of cocoons, frisons, and other waste silk caused by the microorganisms, since the development of the latter is substantially checked by the application of CS_2.

An effective application is at least $250_{cc.}$ for one cubic meter of air, because $200_{cc.}$ is necessary for S bacillus, $250_{cc.}$ for M bacillus and $160_{cc.}$ for P. brevicaule.

We must now consider whether such chemicals as CS_2 and chloroform have any bad influence on silk-fibres. I have investigated this subject a little obtaining the following results:

(1) Experiment with raw silk of 14 denier.

Division of experiment	Quantity applied (*cc*)	Tenacity (*mgr*)	Elasticity (*mm*)	Color and lustre
Fumigated with CS_2.	0.16	52.9	162.0	Sometimes became slightly green.
Fumigated with chloroform.	0.70	56.7	170.4	Normal.
Control.	.....	48.5	177.6	〃

(2) Experiment with raw silk of 16 denier.

Division of experiment	Quantity applied (*cc*)	Tenacity (*mg*)	Elasticity (*mm*)	Color and lustre
Fumigated with CS_2.	0.16	56.4	205.0	sometimes became Slightly green.
Fumigated with chloroform.	0.70	66.1	217.4	Normal
Control.	.....	57.2	195.6	〃

Note: Figures for tenacity and elasticity are average of 30 tests.

From these results, it may be depended on that there is no bad effect on the tenacity and elasticity in consequence of fumigation with CS_2 or chloroform, but sometimes raw silk fumigated with CS_2 assumed a pale green shade.

E. Application of Preventative Methods.

(a) For cocoons.

The best preventative methods applicable to cocoons are probably the following :

(1) The rust on cocoons, as we have seen, is due to the action of microorganisms and such injury occurs, as the rule, under the condition of high temperature united with great humidity. It is therefore necessary, in order to prevent discoloration, to keep the atmosphere of the room where cocoons are spun, at a proper temperature and also free from moisture, that the cocoons may be kept as dry as possible.

(2) Live cocoons contain much moisture, and when stored in piles in warm ill-ventilated apartment, they are apt to become discolored in consequence of reproduction of the microorganisms. Therefore, we must aim to keep the cocoons dry and to store in thin layers at a low temperature.

(3) The processes of stiffling and drying cocoons must be conducted in a thorough manner, especially in case they are going to be stored for a long time, because they will be invaded by moulds unless kept well dried.

Moreover, the drying process should be repeated when stored cocoons show a tendency to be attacked by moulds.

(4) Intrusion of moisture must be prevented absolutely while cocoons are in storage, because if moisture is absorbed it supplies a favourable condition for development of the bacteria and fungi.

(b) For raw silk.

The following precautions are necessary in order to avoid the brown discoloration of raw silks caused by the microorganisms :

(1) In reeling we should not mix healthy cocoons with putrid or mouldy ones.

(2) We should never use stale, dirty, turbid reeling or boiling water.

(3) Let raw silk wound on the small reel be dried as quickly as possible, especially is the utmost precautions needful in case of high temperature and great humidity.

(4) The re-reeling process may be necessary in such a moist climate as that of Japan, since the raw silk wound on the small reel is apt to develop the brown discoloration from the bacteria which flourish best in a moist condition.

(5) In storing raw silk, the moisture must be thoroughly expelled by previous drying and the silk stored in a well-dried place or a closed box.

(c) For boiled cocoons left resting between reeling operations.

In order to prevent the deterioration and discoloration of boiled cocoons left after reeling, the following precaution may be taken :

(1) Keep the cocoons at a low temperature (below 10°C.) to avoid development of the parasitic microorganisms.

(2) Immersion in cold water.

(3) Immersion in water containing antiseptics.

(d) For frisons and other waste silks.

(1) For reeling, healthy cocoons should never be mixed with mouldy or

putrid ones; a careful selection of cocoons should be made for this purpose.

(2) Frisons and other waste silks produced from mouldy or putrid cocoons, should be rinsed as clean as possible in water so as to get rid of the pupae and other matter adhering to them which serve as nutriment to the microorganisms.

(3) Frisons and other waste silks may be boiled in water, for the purpose of destroying the microorganisms adhering to them.

(4) The drying of frisons and other waste silks should be conducted in the quickest manner possible in order to prevent the development of the microorganisms; that is to say, they should be dried by artificial heat or by exposure to direct sunlight, and never be left to dry naturally in shade, especially in a moist and hot climate.

(5) Frisons and other waste silks must be guarded with the utmost care from intrusion of moisture while in storage.

(6) Acid clay may be employed to keep frisons and other waste silks dry while in storage.

(7) Stored frisons may be re-dried at intervals to guard them from becoming discolored.

(8) Fumigation with CS_2, chloroform, etc. is effective, to some extent, for hindering the development of the microorganisms and the consequent brown discoloration of frisons and other silk-fibres.

(9) Immersion in cold water might be adopted for storage of a short period.

Summary.

The main results obtained in my investigations are as follows:

(1) The silk-fibre of cocoons, raw silk, frisons, etc. occasionally assumes a rusty brown tinge, which causes serious loss in the silk industry; for example, a) the so-called "rusty cocoon" is produced among cocoons spun on straw if they have not been well protected from moisture; b) cocoons, especially live ones, develop the discoloration because of having been stored in piles; c) moist

frisons and boiled cocoons left in intervals of reeling, in general, turn brown gradually unless kept at a low temperature; d) raw silks are apt to become discolored if they have not dried quickly during the reeling and re-reeling processes; e) raw silks sometimes assume a brownish tinge if they have not been properly cared for while in storage and they have absorbed much moisture; f) frisons and other waste silks generally brown more or less in the course of the drying and storing.

(2) I have ascertained in my researches that the discoloration of silk-fibre is to be attributed mainly to the action of the microorganisms, although the silk-fibres, in some cases, is spoiled by the use of unclean turbid cooking and reeling water or by the use of otherwise unsuitable water employed in filature processes, such as that containing heavy metals in solution, or by the adhering of fats, oils, and other deleterious substances.

(3) From the discolored cocoons, frisons, and other silk materials used in my experiments, I have, in fact, in every case, without exception, been able to isolate certain species of bacteria and fungi.

(4) There are two kinds of bacteria, S and M, which cause the brown discoloration of silk-fibres; the former belongs to the Bacillus subtilis group, and the later to the Bacillus mesentericus group. Both are common and widely distributed everywhere but their main source is the straw in which the cocoons are spun.

The bacteria from the straw first infect the cocoons and cause their discoloration through rapid reproduction in the moist, warm atmosphere. Moreover, these bacteria, producing heat-resisting spores, survive the effect of high temperatures during the stiffling, drying, cooking and reeling of the cocoons and continue to thrive on raw silks, boiled cocoons left after reeling, frisons, etc. causing the brown discoloration.

(5) 8 kinds of fungi, belonging to the genera of Aspergillus and Penicillium, i. e., A. glaucus, A. glaucus var. α, A. glaucus var β, A. flavus, A. fumigatus, A. albus, P. commune, and P. brevicaule, have been detected in my experiments as the chief species of cocoon fungi; among these, P. brevicaule, A. glaucus, and A. flavus are the most common and harmful. They

grow best on the pupa of the cocoon, and injure its silk-layers ; they attack, moreover, especially P. brevicaule, frisons and other waste silks, causing the brown discoloration.

(6) The reason of the brown discoloration of silk-fibres infected by the microorgamisms is the production of a brown pigment (called melanin) through the oxidation of tyrosin by tyrosinase which is found in the microorganisms, and the tyrosin is derived from the decomposition of the protein substances, especially sericin, of the silk, through the proteolytic action of an enzyme secreted from the microorganisms.

(7) The silk-fibres, being attacked by the microorganisms, produce, as a final decomposition product, ammonia gas which is clearly detected by use of red litmus-paper.

(8) Discolored silk-fibres are apt to be inferior in quality and strength.

(9) In order to prevent the brown discoloration of silk-fibres caused by microorgamisms, we should take precautions to stop their development in the following ways : a) by drying materials as thoroughly and as quickly as possible before storing ; b) by keeping materials from absorbing moisture while in storage ; c) by keeping materials at a low temperature so that the microorganisms are not able to develop ; d) by using clean cooking and reeling water ; e) by keeping materials (such as frisons) under water with the aim of preventing the development of those aerobic microorganisms ; f) by using chemicals, for example, using carbon bisulphide for fumigation; and so on.

In short, my recent investigations have established the fact that the rusty brown discoloration of silk-fibres, the cause of which has hitherto not been well understood, is produced by bacteria and fungi, and that the discoloration can be avoided by preventing the development of those microorganisms.

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EXPLANATION OF PLATES

PLATE I.

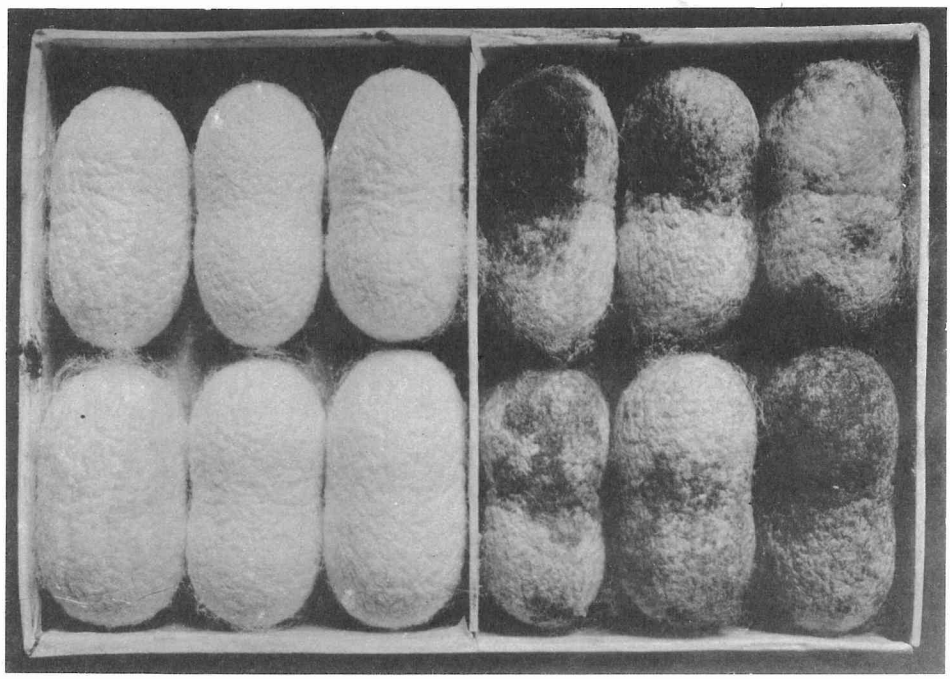
A. 1. Normal white cocoons.
2. Mouldy cocoons.

B. 1. Normal white frisons.
2. Discolord frisons.

PL. I.

A

1 2



B

1 2

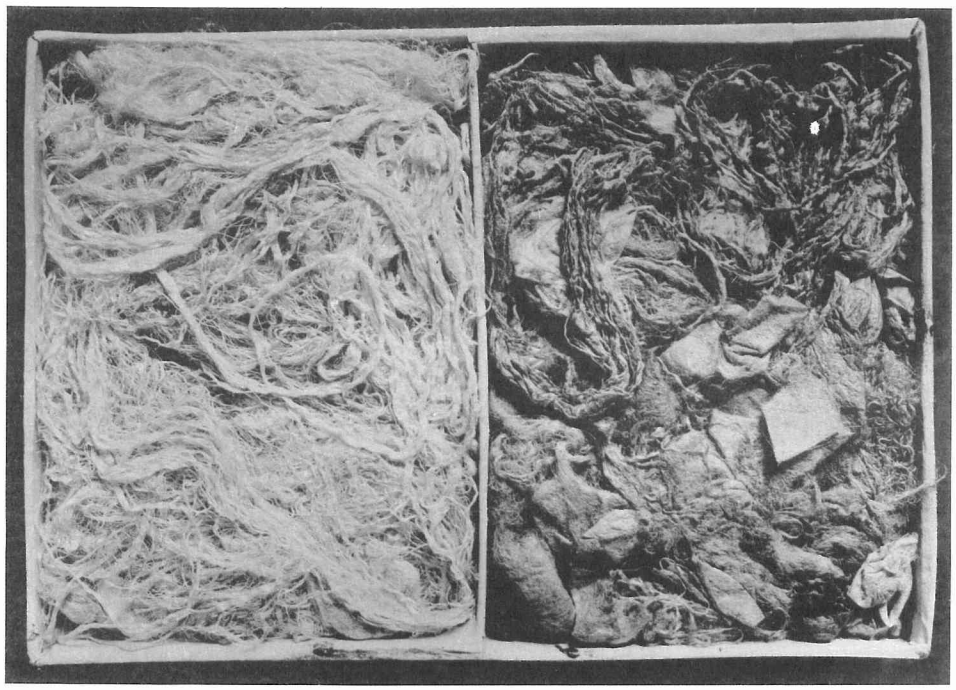


PLATE II.

A. Rusty brown discoloration due to the bacteria.

1. Cocoons discolored as result of inoculation with S bacillus. (After 3 days at 30°C.)
2. Cocoons discolored as result of inoculation with M bacillus. (After 3 days at 30°C.)

B. Sericin culture. (After 3 weeks at 27°C.)

1. Control.
2. Inoculated with M bacillus.
3. Inoculated with S bacillus.
4. Inoculated with P. brevicaule.

C. Culture in egg-albumen.

1. Control.
2. Inoculated with M bacillus.
3. Inoculated with S bacillus.
4. Inoculated with P. brevicaule.

D. Agar slant culture with tyrosin added. (After 18 days at 30°C.)

1. Control.
2. Inoculated with M bacillus.
3. Inoculated with S bacillus.

PL II.

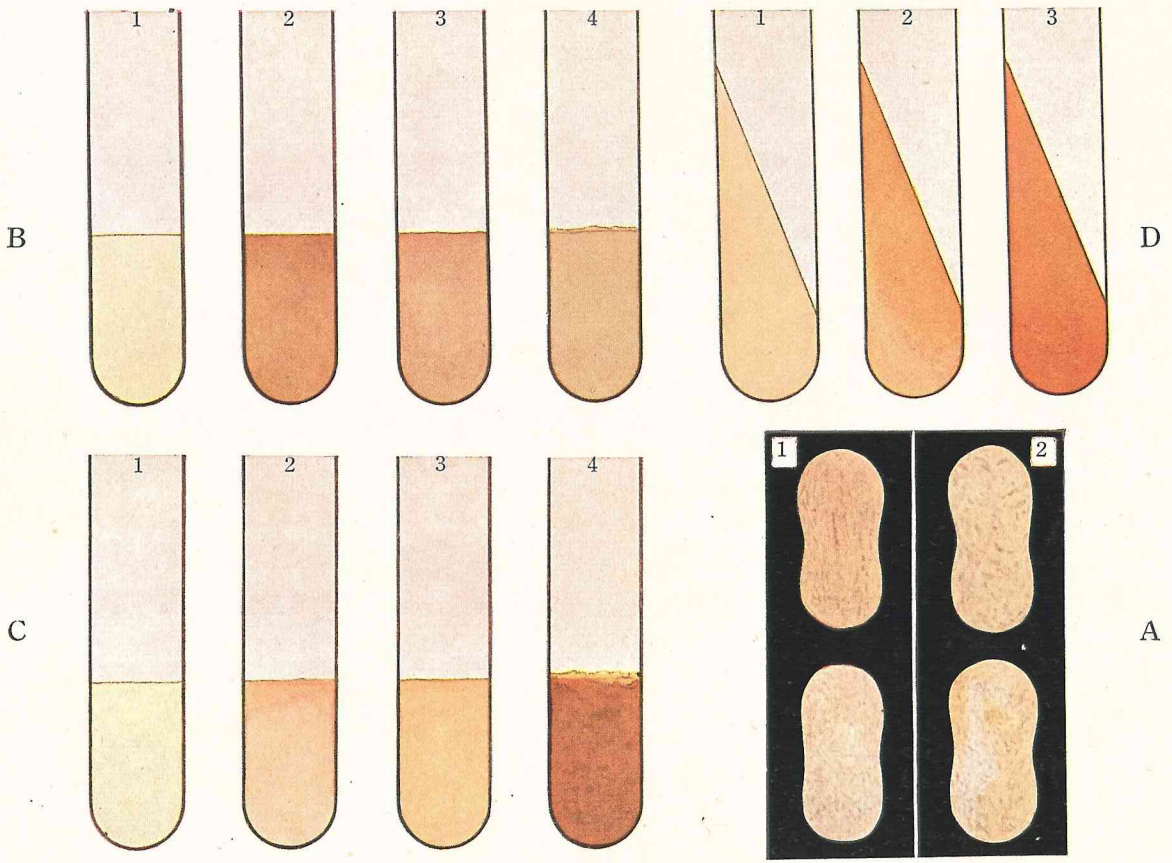


PLATE III.

A. Photograph showing the bacterial discoloration of cocoons. (Bivoltinous "Ohkusa")

1. Discolored cocoons containing no Pupa ; condition after 3 days, having been moistened and incubated at 27°C.
2. Normal white cocoons.

B. Photograph showing the bacterial discoloration of cocoons. ("Shokei")

1. Discolored cocoons containing no Pupa; condition after 3 days, having been moistened and incubated at 27°C.
2. Normal white cocoons.

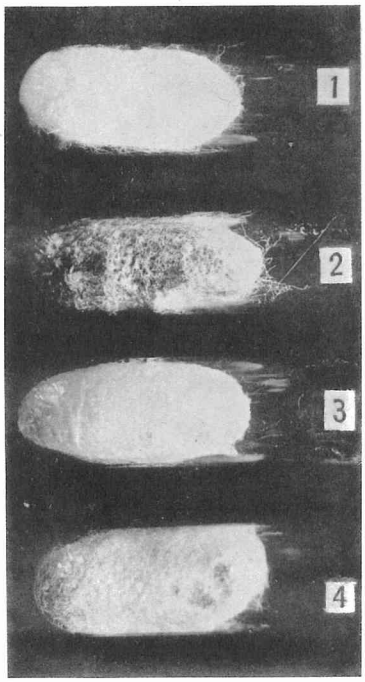
C.

1. Normal white cocoons.
2. Cocoons discolored as result of inoculation with S bacillus; condition after 5 days at 30°C.
3. Inoculated with M bacillus; the same condition as above.
4. Inoculated with P. brevicaule. ; the same condition as above.

D. Gelatin stab cultures of S bacillus isolated from different sources ; condition after 2 days at 19°C.

1. From frison.
2. From white cocoon.
3. The same.
4. From rice-straw.
5. The same.

PL.III.



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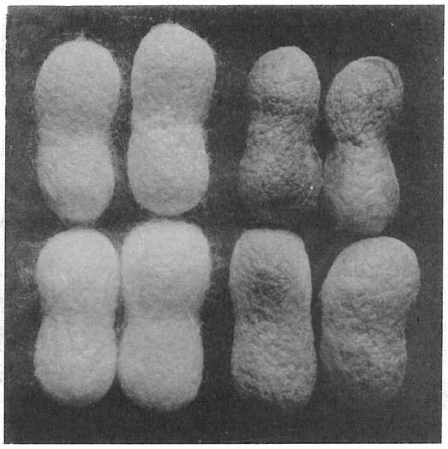

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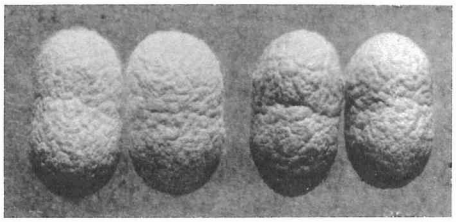


B

D

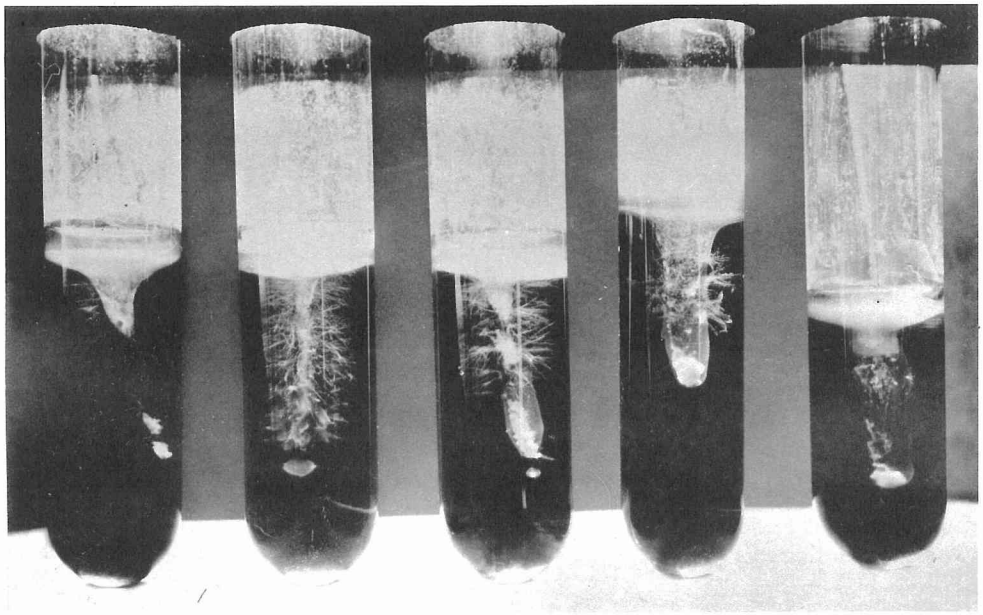
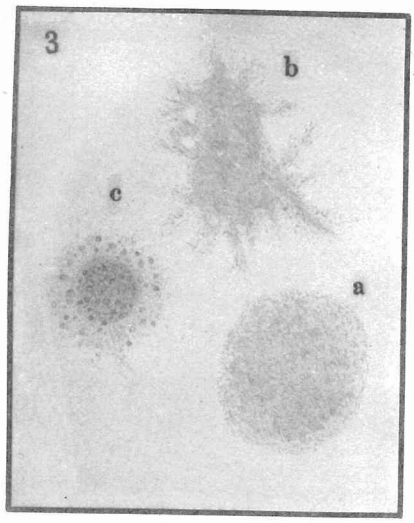


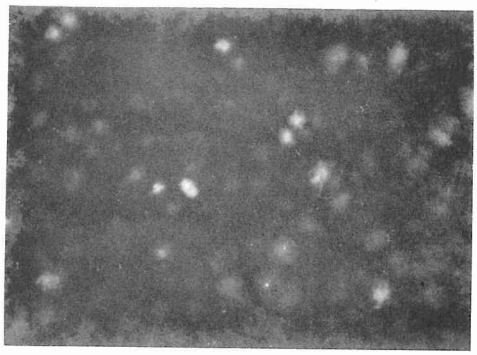
PLATE IV.

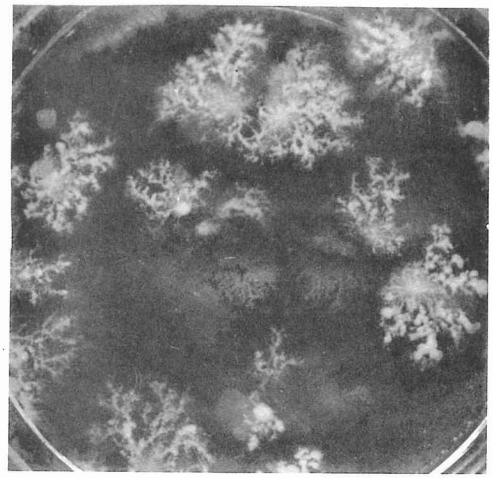
S bacillus.

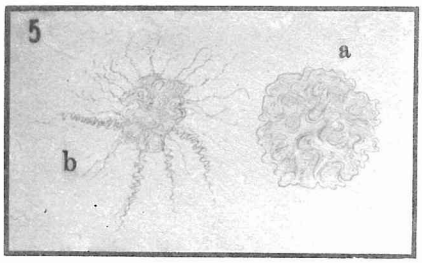
1. Buried and surface colonies on a rather thickly sown agar plate. (After 48 hours at 25°C.)
2. The same on a thinly sown plate.
3. Agar colonies. (After 48 hours at 24°C.) ×16.
   a. Surface colony.
   b. Bottom colony.
   c. Colony situated immediately below the surface.
4. Buried and surface colonies on gelatin plate. (After 3 days at 19 C.°) ×16.
   a. Surface colony; inside of the circular line showing liquefaction of the medium.
   b. Buried colony.
5. The same as 4. (After 5 days at 19°C.) ×16.
   a. Surface colony.
   b. Colony provided with fin-like and hair-like outgrowths.
6. Potato culture. (After 1 week at 27°C.)

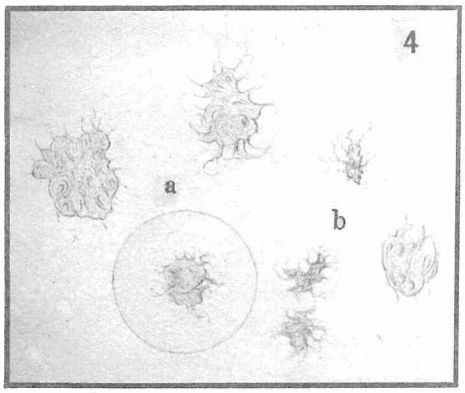
PL. IV











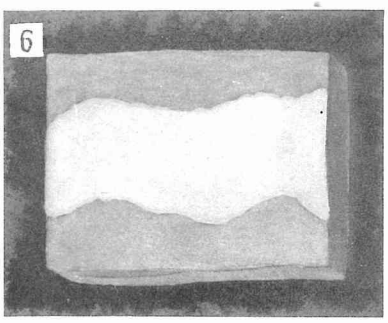
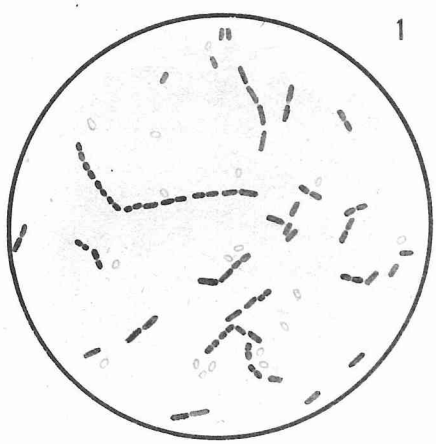


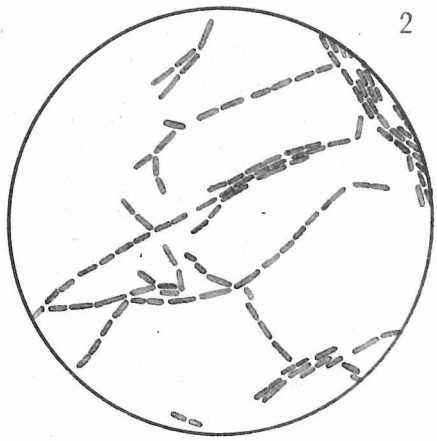
PLATE V.

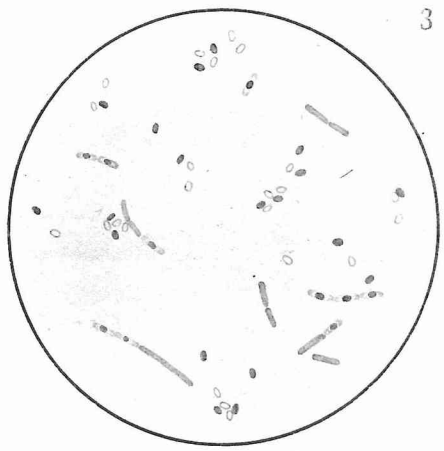
S bacillus.

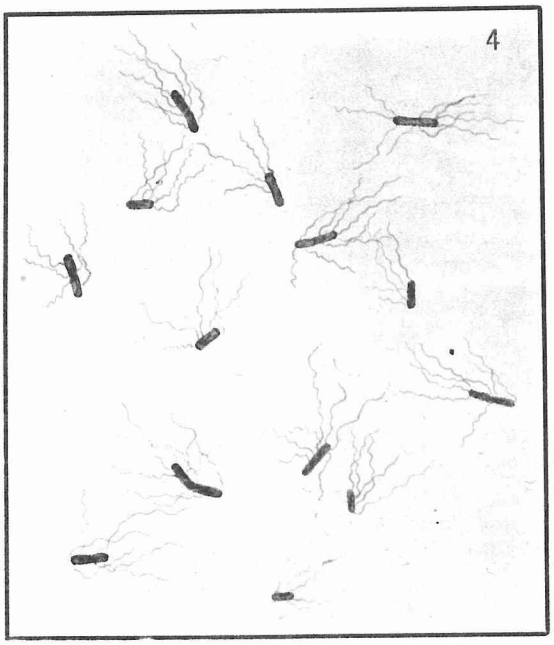
1. Vegetative cells from agar stroke culture at 30°C. for 24 hours. × 800.
2. The same from nitrate peptone culture at 30°C. for 24 hours. × 800.
3. Sporangium and spores stained with carbol fuchsin and methylene blue. × 800.
4. Flagellate rods stained by Löeffler's method. × 800.
5. Agar slant culture. (After 48 hours at 35°C.)
6. Agar stab culture. (The same.)
7. The same; surface colony.
8. Gelatin stab culture. (After 48 hours at 19°C.)

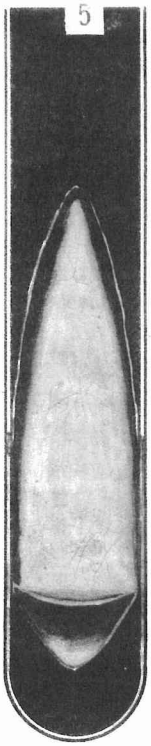
PL. V.

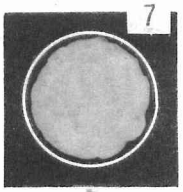












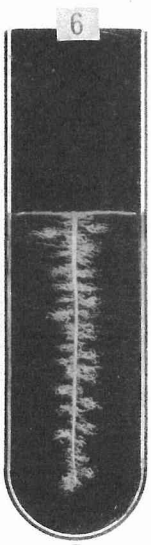


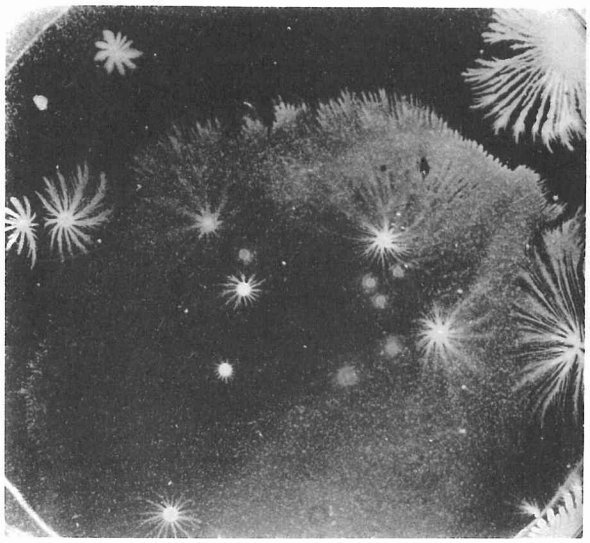
PLATE VI.

M. bacillus.

1. Buried and surface colonies on a rather thickly sown agar plate; this culture was first kept at 28°C. for 17 hours, later at 35°C. for 48 hours.
2. The same on a thinly sown plate.
3. Branched surface colony with finger-like outgrowths. × 2.
4. Amoeboid surface colony. × 8.
5. Round colony with several folds. × 8.
6. Round colony with concentric circular lines. × 8.
7. Bottom colonies. × 8.
8. Buried colonies. × 16.
9. Buried and surface colonies on gelatin plate. (After 3 days at 19°C.) × 8.
    a. Common surface colony.
    b. Surface colony provided with faint anastomosing outgrowths; inside of boundary line showing liquefaction of the medium.
    c. Surface colonies provided with radial rays.
    d. Buried colony.

PL. VI.

2



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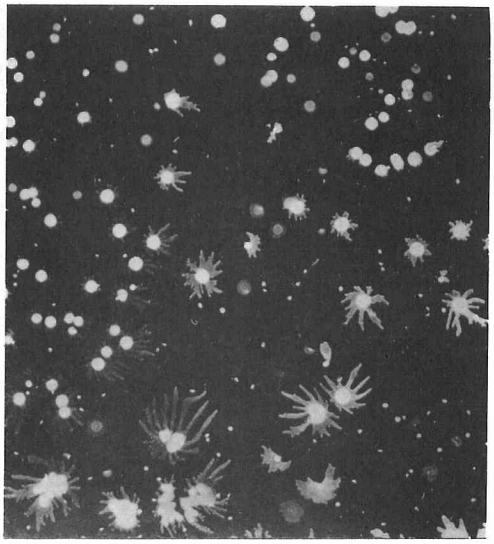
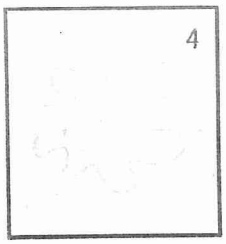
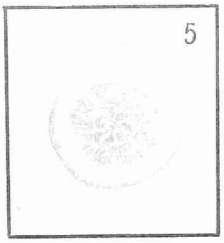
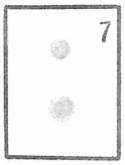
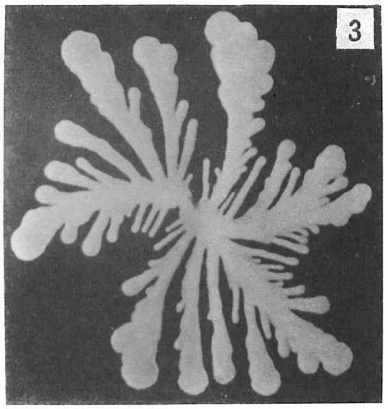
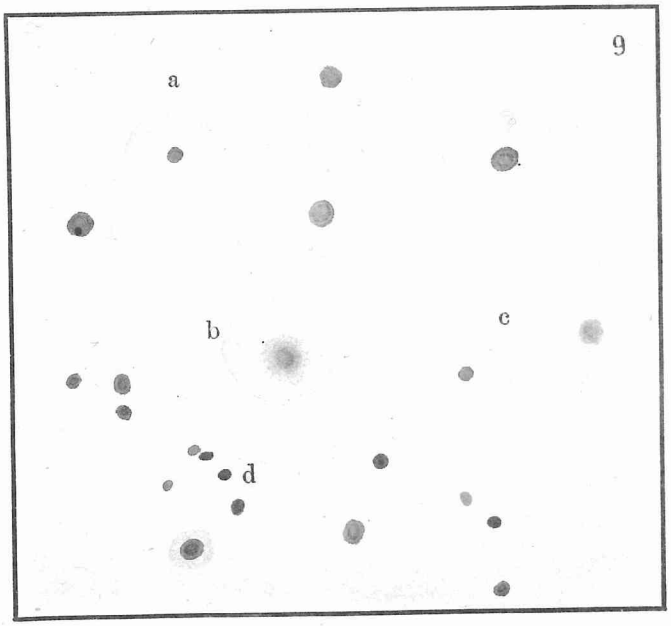


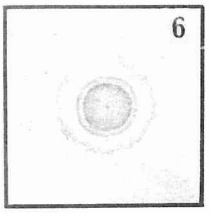












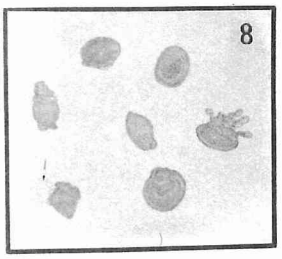
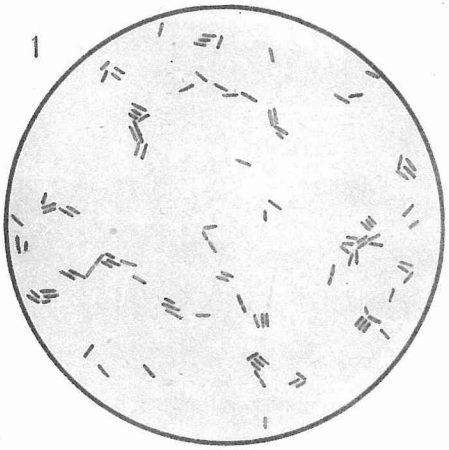


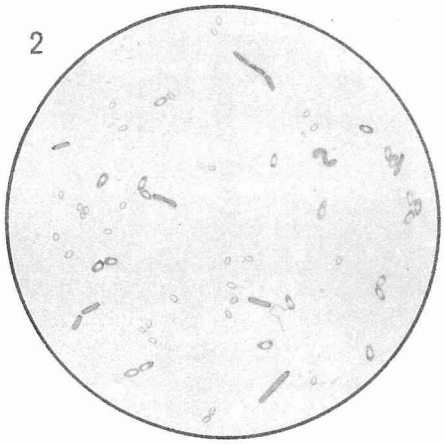
PLATE VII.

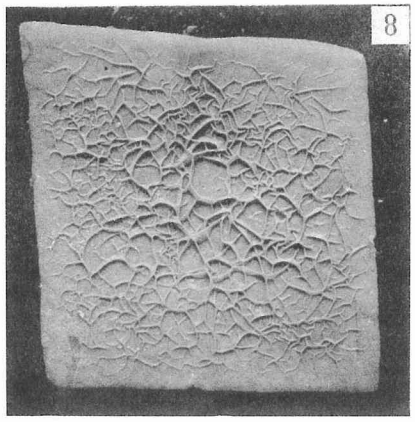
M bacillus

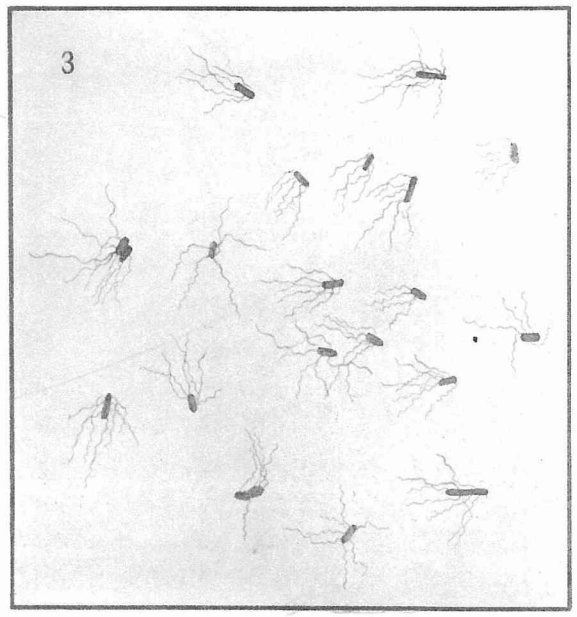
1. Vegetative cells from agar stroke cultivated at 27°C. for 24 hours. × 800.
2. Sporangia and spores. × 800.
3. Flagellate bacilli. × 800.
4. Agar slant culture. (After 48 hours at 35°C.)
5. Agar stab culture. (The same.)
6. The same; surface colony.
7. Gelatin stab culture. (After 48 hours at 19°C.)
8. Potato culture. (After 3 days at 27°C.) × 1,5

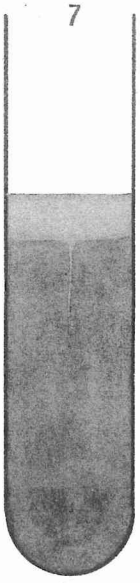
PL. VII.

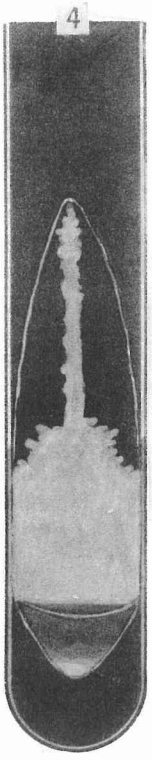


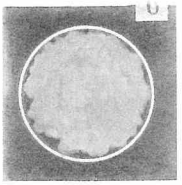












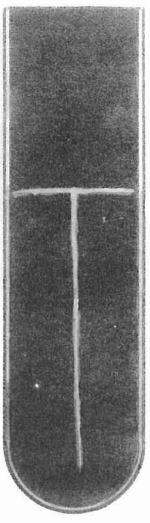


PLATE VIII.

Mouldy cocoons and pupae.

1. Aspergillus glaucus.
2. Aspergillus glaucus. var. α.
3. Aspergillus flavus.
    a. Greenish form.
    b. Yellowish form.
4. Penicillium commune.
5. Aspergillus albus.
6. Aspergillus fumigatus.
7. Penicillium brevicaule.

PL. VIII

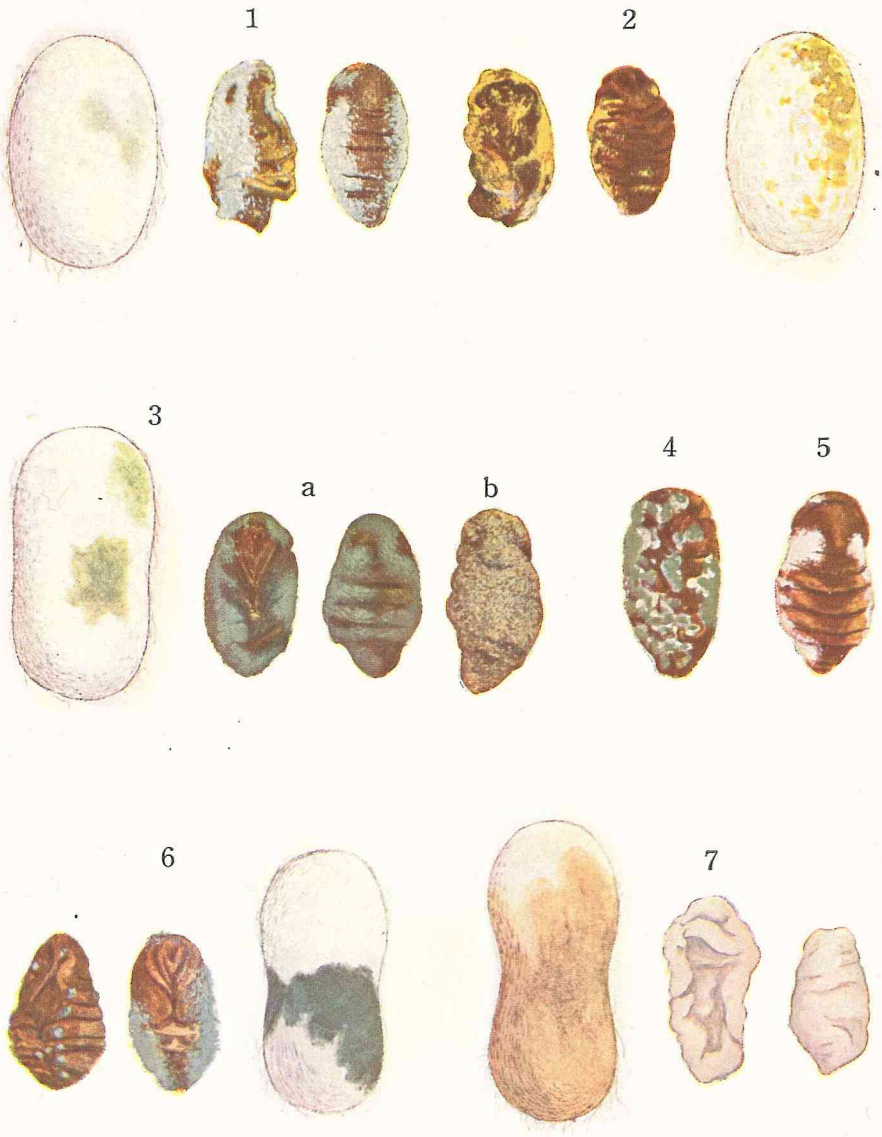


PLATE IX.

Cultures of cocoon fungi.

1. Aspergillus flavus. (Cane-sugar added to agar culture; after 1 week at 22,5°C.)
2. Aspergillus glaucus. (The same.)
3. Aspergilius glaucus var. α. (The same.)
4. Penicillium brevicaule. (The same.)
5. Aspergillus glaucus var. β. (The same.)
6. A. flavus. (Miyoshi's Soya culture; after 6 days at 25°C.)
7. A. glaucus. (The same.)
8. A. glaucus var. α. (The same.)
9. A. glaucus var. β. (The same.)
10. Tyrosin added to agar culture with Penicillium brevicaule, showing a deep brown discoloration of the medium. (After 1 month at 22,5°C.)

PL. IX.

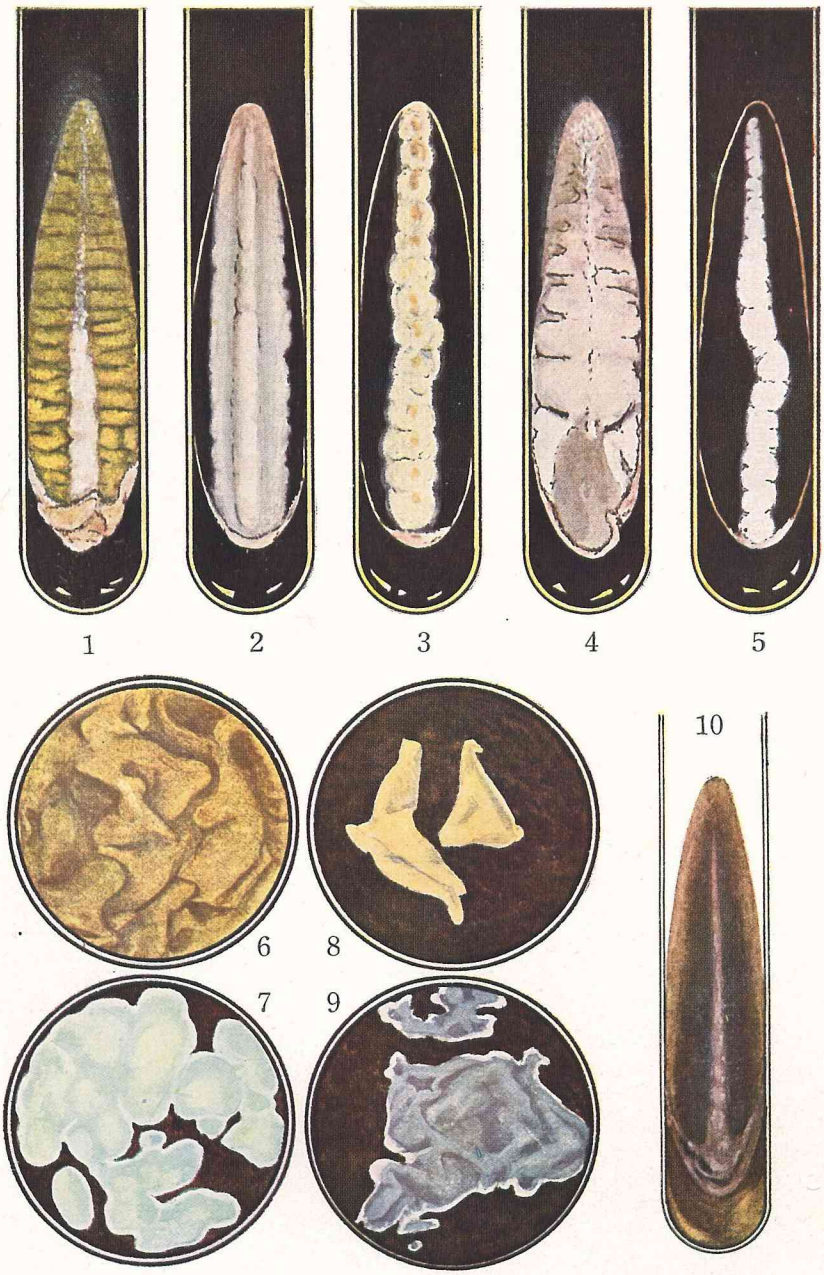


PLATE X.

1. Aspergillus glaucus. (Upper portion of conidiophore produced on Miyoshi's Soya medium.) × 500.
2. The same. (The same produced on pupae.) × 500.
3. The same; conidia. × 800.
4. The same; a colony developed on agar.
5. The same; a perithecium from pupa decoction added to agar culture. × 500.
6. The same; young asci. × 800.
7. The same; mature asci and ascospores. × 800.
8. The same; a group of ascospores free from ascus. × 800.
9. The same; side view of ascospores. × 800.
10. Aspergillus glaucus var. α; a colony developed on agar.
11. The same; upper portion of a conidiophore. × 500.
12. The same; branched abnormal conidiophore. × 500.
13. The same; abnormal conidiophore with septa. × 500.
14. The same; conidia. × 800.
15. The same; vegetative hyphae containing numerous yellow granules. × 800.
16. The same; coiled hyphae which are going to form perithecia.
17. Aspergillus glaucus var. β; upper portion of conidiophore. × 500.
18. The same; abnormal conidiophore. × 500.
19. The same; conidia. × 800.

PL. X.

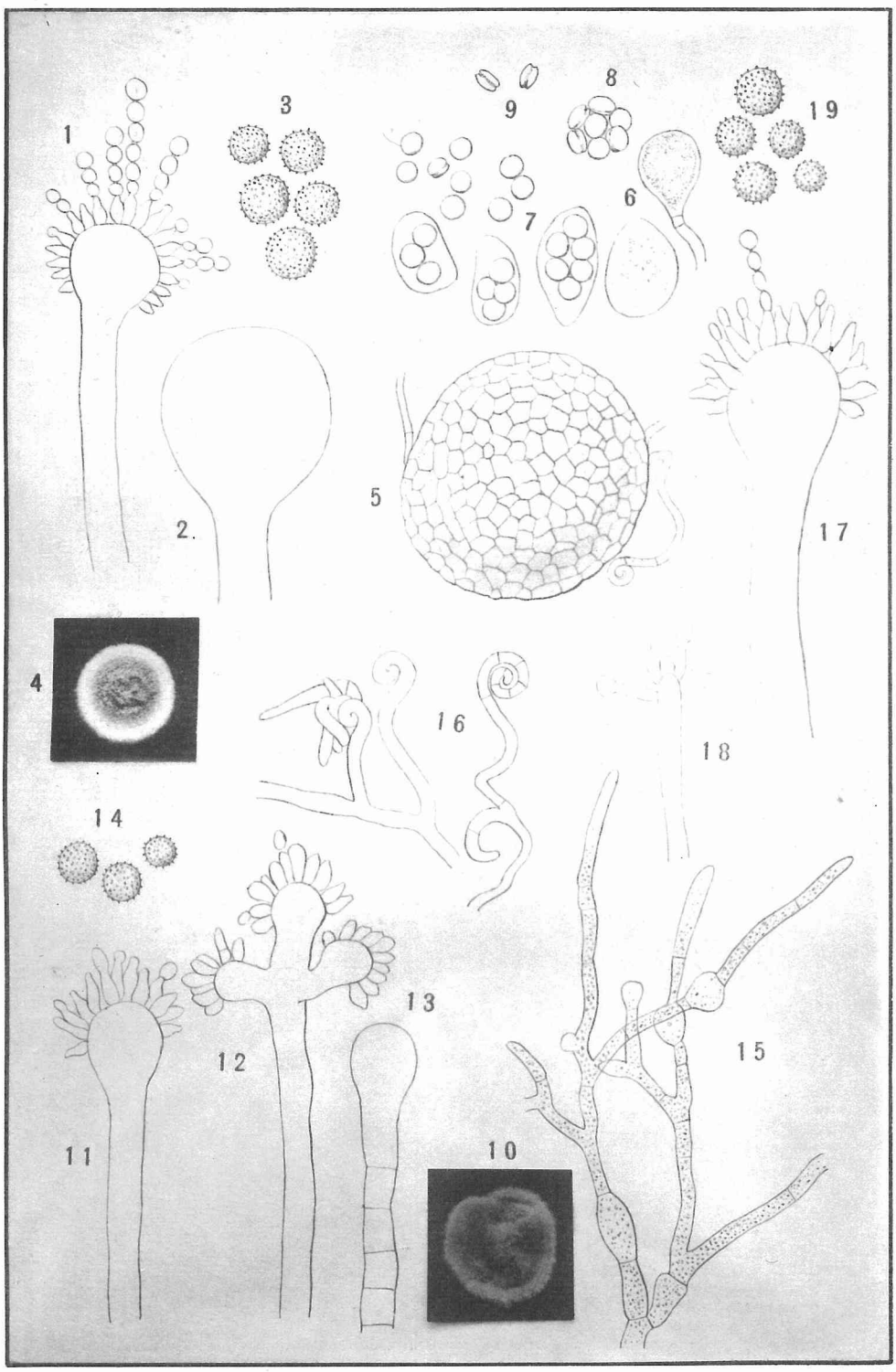


PLATE XI.

1. Aspergillus flavus. A colony developed on steamed potato.
2. The same; conidiophore. × 120.
3. The same; upper portion of conidiophore. × 500.
4. The same; conidia in chains. × 500.
5. The same; conidia. × 800.
6. The same; germinating conidia. × 500.
7. Aspergillus fumigatus. A colony developed on agar plate. × 2.
8. The same; conidiophore. × 500.
9. The same; conidia. × 800.
10. Aspergillus albus. Young colonies developed on steamed potato.
11. The same; upper portion of conidiophore. × 500.
12. The same; conidia. × 800.
13. Penicillium commune. Two colonies developed on steamed potato.
14. The same; conidiophore. × 500.
16. The same; conidia. × 800.
17. The same; vegetative hyphae and small conidiophores. × 500.
18. The same; abnormal conidiophores. × 500.

PL. XI.

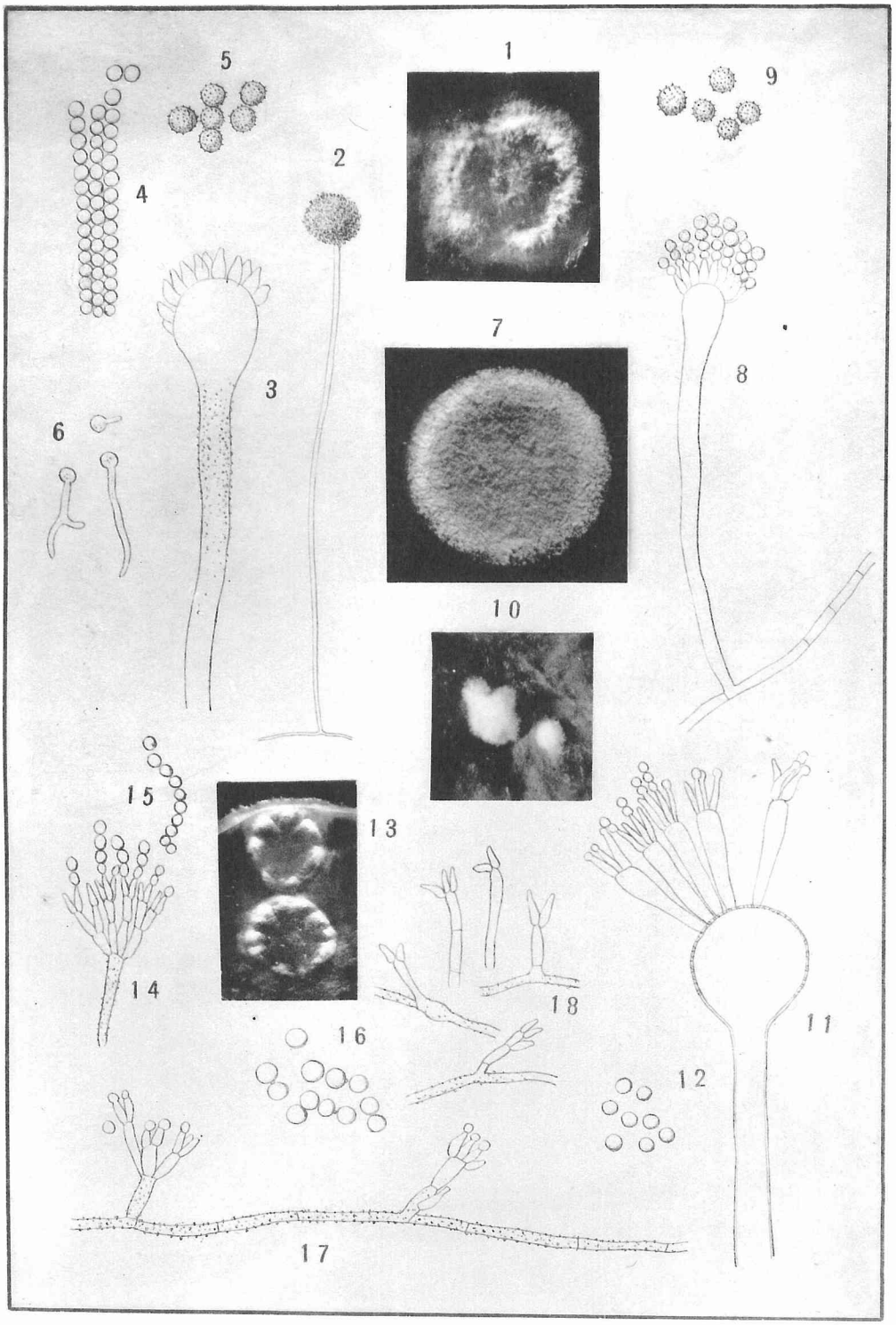


PLATE XII.

Penicillium brevicaule.

1. Conidiophores and conidia obtained from mouldy cocoons. × 800.
2. Conidia in optical section. × 800.
3. Conidiophores and conidia developed on nitrate peptone medium. × 800,
   (The red granules show oily substances stained with Sudan III.)
4. Vegetative hyphae developed in the same medium. × 800.
5. Simple conidiophore. (From bread culture.) × 800.
6. Conidiophore with two sterigmata. (The same.)
7. Conidiophore with three sterigmata. (The same.)
8. Conidiophore with many sterigmata. (The same.)
9. Dwarf conidiophore. (The same.)
10. Catenulate conidia. × 800.
11. Conidiophores and conidia developed on starch media. × 800.
12. Vegetative hyphae developed in the same medium. × 800.
13. Part of hyphae from the white mycelial mass grown on agar slant. × 800.
14. Mycelial bundles and conidiophore grown on steamed potato. × 800.

PL. XII.

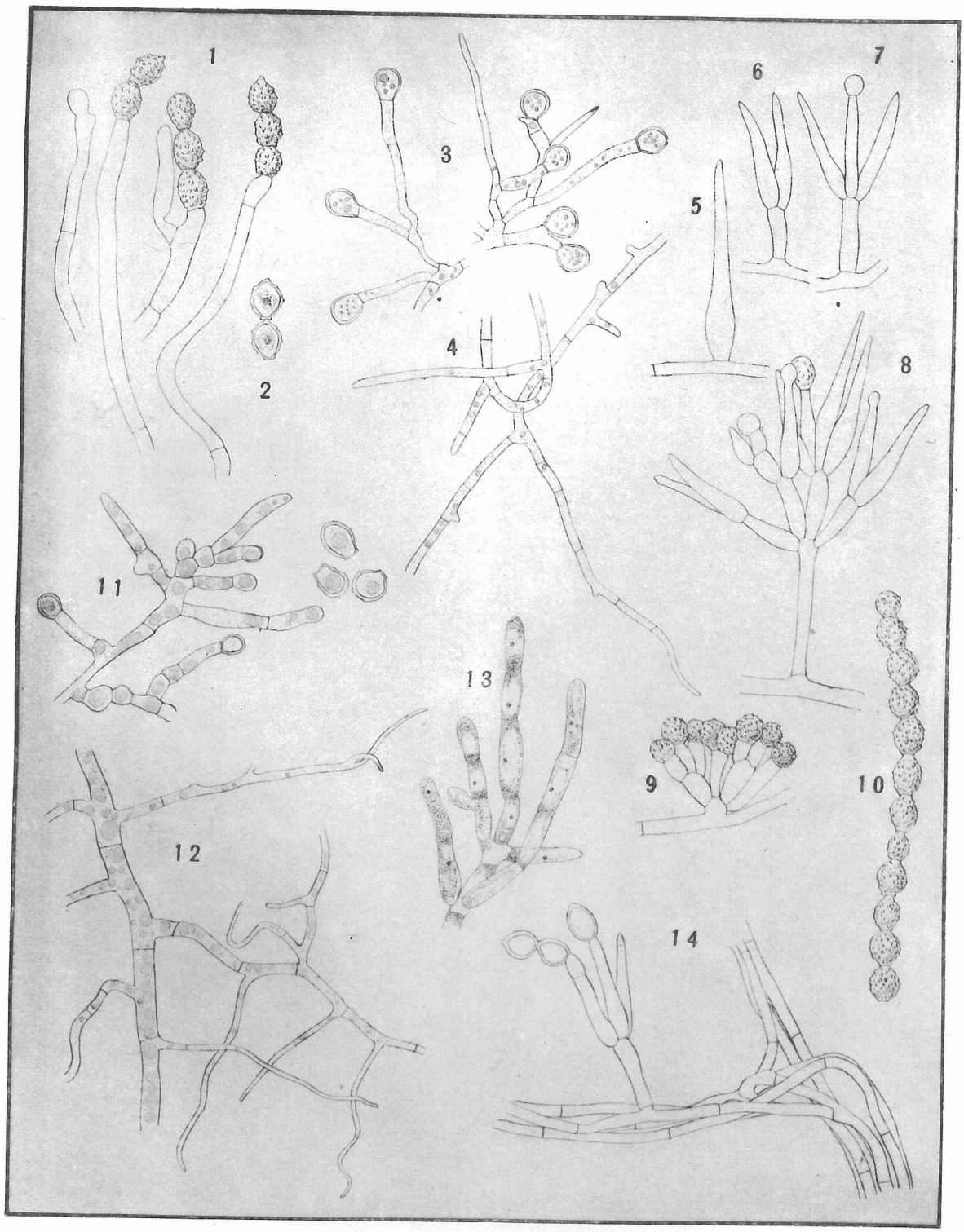


PLATE XIII.

Penicillium brevicaule.

1. Potato culture. (after 3 days at 22,5°C.)
2. The same in more advanced stage. (After 1 week.)
9. The same. (After 2 weeks.)
4. The same. (After 3 weeks.)
5. Culture with egg-albumen. (After 16 days at 30°C.)
6. Culture with nitrate peptone medium. (After 16 days at 30°C.)
7. Frison inoculated with P. brevicaule ; condition after 10 days at the ordinary temperature in June.

PL. XIII.