The Action of Copper and Manganese upon the Formation and Color of Conidium of Some Species of *Aspergillus*

By

Miss FUJI YOSHIMURA

(With Plates IV–IX and 37 Text-figures)

Introduction

It is a well known fact, that the sporulation of many fungi is influenced by several conditions, among which the action of some heavy metals may be mentioned as important. In this connection the following works are to be consulted.

Fe[1], Mn[2], Cu[3] generally, and sometimes Zn[4] stimulate the sporulation, while Cd[5] and sometimes Zn[6] act to inhibit it. The inhibiting action of Zn and Cd can be completely or partially overcome by the addition of Fe, Mn or Co[7].

For the development of the proper color of conidium of *Aspergillus niger*,[8] *Asp. oryzae*[9] and *Asp. flavus*[10] it is necessary to add Cu. In *Aspergillus niger* brown or gray conidia are produced by Zn[11] or Ba[12], and the color of the conidium of *Asp. oryzae* as well as

1) GOLLMICK (1936), SAUTON (1911, 1913) and BERTRAND (1912).
2) BERTRAND (1912) and LAPPALAINEN (1919).
3) WOLFF and EMMERIE (1930) and GOLLMICK (1936).
4) BERTRAND (1912), METZ (1930) and SAUTON (1913).
5) GOLLMICK (1936), LEPIERRE (1913) and BORTELS (1927).
6) GOLLMICK (1936).
7) BERTRAND (1912) and GOLLMICK (1936).
8) RICHARDS (1897), STEINBERG (1919), BORTELS (1927), ROBERG (1928), YOSHIMURA (1934) and GOLLMICK (1936).
9) and 10) MCHARGUE and CALFEE (1931).
11) RICHARDS (1897) and PORGES (1932).
12) LAPPALAINEN (1919).

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of *Asp. flavus* is changed from yellowish green to pale green or to white by failure to add Mn[1].

The conidium formation and color development of Aspergilli also have some connections with the age of the culture[2] and with the acidity of the culture medium[3]. In the case of *Penicillium glaucum* they are concerned with the combination of several heavy metals[4].

The formation of an abnormal conidium-bearing head has been reported by several authors, who believed that it is connected with the kind of culture solution[5], nitrogen source[6], omission of K[7], high humidity[8] or high temperature[9]. WEHMER (1913) has found a morphological change of conidium caused by the submersion of mycelium in the culture solution.

In the present paper the writer has attempted to report her experimental results on the combined action of Cu amd Mn upon the sporulation and on the rôle of Cu in respect to the color development of conidium in some species of *Aspergillus*.

The composition of the culture solution was as follows:

NH_4NO_3	5 g
KH_2PO	2.5 g
$MgSO_4.7H_2O$	1.25 g
Glucose	22.5 g
Redistilled water	1000 cc.

The pH-value of the solution was adjusted by the addition of NaOH to 5.5. In order to remove heavy metal impurities in the culture solution the adsorption procedure was practiced, treating the solution with 0.5% Ca-phosphate, according to the method described in detail in the work of SAKAMURA (1936). As culture vessels 250 cc. ERLENMEYER flasks of Terex glass were used; 100 cc. of the culture solution were placed in each flask and sterilized exactly for

1) McHARGUE and CALFEE (1931).
2) TAKEDA (1910) and BLOCHWITZ (1928, 1931).
3) MEDISH (1910) and BLOCHWITZ (1928, 1931).
4) HAENICKE (1916).
5) BEAUVERIE (1900), ZIKES (1924) and KARDO-SEYSSOJEWA (1936).
6) LUTZ (1905).
7) MOLLIARD et COUPIN (1903).
8) KLEBS (1896, 1898) and BLOCHWITZ (1925).
9) MANGIN (1908).

20 minutes in a KOCH steam sterilizer. To the culture solution, which was treated with Ca-phosphate, Fe (2×10^{-6}mol) and Zn (10^{-6}mol) in the form of sulphate were added and the solution thus prepared was called "standard solution". The conidia used for the inoculation, were taken from an agar-agar culture which contains the standard solution[1] plus Mn (10^{-6}mol).

Experiment 1.

To the standard solution were added Cu (10^{-6}mol) and Mn (10^{-6}mol) in the form of sulphate and the influence of these heavy metals upon the conidium formation was investigated. Duration of culture: 7 days.

Table 1

Aspergillus niger

Heavy metal added	pH	Conidium	Remarks
—	2.2	± ivory buff	mycelium felt smooth
Cu	2.0	+ black	felt folded and discontinuous
Mn	2.2	± ivory buff	felt smooth
Cu+Mn	2.1	+++ black	felt smooth

Table 2

Aspergillus oryzae

Heavy metal added	pH	Conidium	Remarks
—	3.1	± yellow ocher	felt smooth
Cu	3.2	—	felt folded, mostly submersed in solution, spherical cells.
Mn	3.0	± yellow ocher	felt smooth
Cu+Mn	2.3	+++ olive ocher	felt smooth

1) The concentration of glucose was reduced to 1/10.

Table 3

Aspergillue flavus

Heavy metal added	pH	Conidium	Remarks
—	2.4	± Naples yellow	thin film of felt
Cu	2.4	± Naples yellow	,,
Mn	2.4	+ Naples yellow	felt smooth
Cu+Mn	1.8	++ sulphine yellow	,,

From these results it will be seen that Cu plays an important rôle upon the sporulation and the development of conidium color. In the case of *Aspergillus niger* the addition of Cu acts to stimulate the conidium formation, but this is not true in *Asp. oryzae*, where the spherical cells are formed instead of conidia. Mn has no direct influence upon the sporulation, but keeps the mycelial development in the normal state, because it interferes with the spherical cell formation. This results indirectly to benefit the sporulation. The same thing can apply to Fe and Co, both of which hamper the spherical cell development. Their concentration, which is necessary to induce the growth of the normal mycelium, must be, however, higher than that of Mn.

Development of abnormal conidium-bearing head caused by the presence or absence of Cu and Mn

From the results of the experiment with *Asp. oryzae* mentioned above, it is learned that the addition of Cu and Mn together in the culture solution is necessary for the normal development of conidium and failure to add any one of them results in a diminution in the sporulation. In the last case some abnormal conditions are recognizable also on the conidial head, even with the naked eye. The omission of Cu causes a reduction of the number of stalks and conidia and a diminution in the head development. If Mn is omitted, stalks are remarkably shortened in *Asp. niger* and *Asp. flavus* and almost no sporulation happens in *Asp. oryzae*. In the following experiment detailed microscopical observations were carried out and many

microphotograms will be shown in order to make clear the explanation.

Experiment 2.

The culture was conducted in the same manner as in the foregoing experiment and continued for 5–8 days.

Aspergillus niger: The addition of Cu and Mn together results in the development of intense felts on which normal conidia are produced. If Cu is omitted, the conidia are ivory buff, stalks are diminished either in the diameter or in the length, and heads are of very small size. Sterigmata or cells forming conidia sometimes swell spherically or lengthen in the mycelial form (Photo 1, 2). Omission of Mn and increase of the quantity of Cu (5×10^{-3}mol) causes rare development of conidia and often a shortening and branching of the stalks (Photo 3).

Aspergillus oryzae: In the presence of Cu and Mn together the mycelial growth goes on vigorously and the sporulation occurs normally (Photo 4). In the case of the addition of Cu alone the mycelial felt is submerged in the solution and small parts of it appear here and there above the surface of the solution. Macroscopically no conidium formation is ascertained, but microscopically it is seen to occur rarely with heads very abnormally formed. By addition of Mn even in a trace (5×10^{-8}mol) the production of abnormal conidia is promoted, and the observation of the conidial heads thus formed permits the investigation of several transition forms between conidia and spherical cells.

In the first stage of the abnormal formation of heads many normal conidia are also found on the head, while some of the sterigmata are elongated (Photo 5) or their ends are swollen (Photo 6, 7, Fig. 1). In the most abnormal case no normal conidium is formed on the head, but colorless spherical cells are produced at the end of the elongated sterigmata, sometimes without vesicle (Photo 8) and sometimes with a normal one (Photo 9 and 10). In these cultures many spherical cells are formed on the surface of the solution or in it (Photo 11, 12, Fig. 2, 3, 4, 5).

The action of Cu, benefiting the spherical cell formation, is not limited to the ordinary mycelium, but extends to the end of the stalk growing in the air space and transforms sterigmata and conidia into spherical cells. An addition of Mn (10^{-7}mol) to the Cu-con-

taining solution reduces the abnormality of the head formation, and by a larger increase of the quantity of Mn (10^{-6} mol) the fully normal condition of the conidial formation is recovered. The stalk cell can be distinguished from the mycelial cell by its larger diameter, thicker membrane and the special staining affinity to methylene blue. Some of the spherical cells are also of thick membrane and easily stainable with methylene blue. Such a spherical cell is rich in contents, some of which shows a blue color reaction with iodine (Photo 11, 12, Fig. 5). Many spherical cells grow secondarily from the primary ones, which phenomenon bears a striking resemblance to the development of sterigmata and conidia from vesicles (Photo 11, Fig. 2, 3, 4, 5). Some of the stalks and vesicles are of thick membrane and show a blue color reaction with iodine, as do the spherical cells (Fig. 6).

Conidia are produced not only in the stalks, but sometimes come out also at the axils of the branches of the septate ordinary mycelia (Photo 13). Upon the addition of Cu (10^{-6}mol) and Mn (5×10^{-8}mol) together, such abnormal sporulation is often observed accompanied by the abnormal heads mentioned above.

In the culture to which Mn has been added poor sporulation occurs and the color of the conidia is yellow ocher. Most heads are normally formed, but they are of small size and only two or three conidia grow at the end of each sterigma. Besides such small heads a few abnormal ones appear; for instance, vesicles without sterigma or bearing mycelia instead of sterigmata are found (Photo 14, Fig. 7). If the quantity of Mn is increased (10^{-5} or 10^{-4}mol), a second, third or sometimes forth head is formed (Photo 18, Fig. 8), or sometimes a double head on a stalk is observed (Photo 19, Fig. 9).

In case Cu (10^{-6}mol) and Mn (10^{-6}mol) are together added, the normal sporulation occurs, but a further addition of Cd causes abnormalities again. In a concentration 10^{-5}mol of Cd heads are partially and in 10^{-4}mol all of them are constructed abnormally (Photo 20–23, Fig. 10–13).

Aspergillus flavus: The normal sporulation occurs in case of the combined addition of Cu (10^{-6}mol) and Mn (10^{-6}mol) (Photo 24). Omission of Mn from such a culture causes abnormal sporulation, and many transition stages from the conidium formation to the spherical cell formation are observed. Conidia are formed not limited to the stalks, but they grow on the ordinary mycelia accompanied by the spherical cells (Photo 25–28, Fig. 15–23). Addition

of Mn in the concentration 5×10^{-8}mol is not enough to exclude such abnormalities, except that the formation of the stalk becomes more distinct (Photo 29–36, Fig. 24–28). In an old culture of this kind, some of the cells, which correspond to conidia or are growing in the mycelial form from conidia, have thick membrane (Photo 35, 36). In the case of the omission of Cu the heads are small, but normally formed (Photo 40, Fig. 29). By addition of Zn to such a culture the formation of many secondary sterigmata is caused (Photo 41, 42, Fig. 30–32).

The formation of the abnormal heads in the combined addition of Cu (10^{-6}mol), Mn (10^{-6}mol) and Cd (10^{-5}mol or 10^{-4}mol) occurs similarly as in *Asp. oryzae.*

The abnormalities of the sporulation which are mentioned above, are also visible in *Asp. japonicus, Asp. candidus,* (Photo 45), *Asp. parasiticus* (Photo 46), *Asp. tamarii* (Photo 47), and *Asp. clavatus.*

In a series of experiments described above it is learned that abnormal heads of several kinds are produced by the combined action of Cu and Mn. That similar abnormalities are caused by other factors, has been hitherto reported by some authors, and now is confirmed by the present writer in high temperature (40°C) culture of *Asp. niger, oryzae* and *flavus,* even when Cu (10^{-6}mol) and Mn (10^{-6}mol) both were added to the culture solution (Photo 48, 49). The writer does not, therefore, intend to maintain, that the action of heavy metals is the sole factor which causes the development of abnormal heads. However, so far as the culture is carried out with solutions which contain unexpected heavy metal impurities, they will play a very important rôle in the formation of such abnormal heads. This must be taken into consideration with great care, when the morphological character of the conidium is regarded as of great importance in the classification of fungi.

The formation of abnormal heads in Aspergilli, which occurs sometimes in the culture without the addition of Mn as stated in the above experiments, seems to have some relation to the spherical cell formation. This relation may be diagramatically explained in *Asp. oryzae* (Fig. 37).

Hand in hand with the diminution of the quantity of Mn, sterigmata and conidia, both elongated, appear on stalks mixed with the normal conidia with swollen ends (a–d). Omission of Mn causes the development of many spherical cells in place of conidia on the heads, which are now branched and have no appearance of ordinary vesicles

(e). There are many transition forms from conidium to spherical cell (d–h), and it is sometimes difficult to distinguish the two forms. Such a modification of conidium to spherical cell is remarkable, especially when cells grow near the surface of the culture solution (g, h). As mentioned above, the spherical cell formation is brought about by the action of Cu without interference of Mn, while Zn acts equally in place of Cu, if its quantity is increased. The same tendency can be seen also in the culture of *Asp. flavus*.

In the culture of Aspergilli it is often recognized, that in high acidity the conidium formation is much reduced and many spherical cells are found in the brittle fungus felt. Such tendency is recognized especially when Mn is omitted and sufficient Cu and Zn are present in the culture solution. For the control of the tendency either in the conidium formation or in the spherical cell formation the presence and absence of these heavy metals play an important rôle.

Action of Mn to the culture of high acidity

In general the sporulation of Aspergilli is remarkably reduced in cultures of high acidity and the fungus felt is folded or consists of individual, but partially coalesced colonies. Many spherical cells are found in such felt which is brittle to the touch[1]. TAMIYA (1928) has reported that Mn acts beneficially upon the growth of *Aspergillus* in high acidity and STEINBERG (1935) has ascertained that omission of Mn results in a very similar development of the fungus felt to that stated above. Therefore, it may be expected that the nature of the fungus felt and the pH-limit of the growth in high acidity would be affected by the addition of Mn. The following experiment 4 shows this relation.

Experiment 4.

The culture procedure was almost the same as in the previous experiments. Cu (10^{-6}mol) was added to the standard solution. The pH-value of the culture solutions was adjusted with distilled H_2So_4.

1) SAKAMURA und YOSHIMURA (1933).

Table 4

Aspergillus niger. Duration of culture: 8 days.

Initial pH	No addition of Mn			Addition of Mn (10^{-6} mol)		
	Final pH	Conidium	Dry weight of mycelium (g)	Final pH	Conidium	Dry weight of mycelium (g)
1.2	1.2	—	0[1)]	1.1	—	0.012
1.4	1.4	—	0	1.4	±	0.553
1.6	1.6	—	0.003	1.5	+	0.742
1.8	1.8	—	0.036	1.6	++	0.710
2.2	1.7	—	0.596	1.8	+++	0.825
2.6	1.7	—	0.558	1.9	+++	0.824
3.2	1.7	±	0.676	2.0	+++	0.815
4.2	1.8	±	0.735	2.0	+++	0.844
5.2	1.9	+	0.801	2.2	+++	0.815

Table 5

Aspergillus oryzae. Duration of culture: 9 days.

Initial pH	No addition of Mn			Addition of Mn (10^{-6} mol)		
	Final pH	Conidium	Dry weight of mycelium (g)	Final pH	Conidium	Dry weight of mycelium (g)
2.2	2.2	—	0	2.1	±	0.228
2.5	2.5	—	0	2.0	++	0.400
3.0	3.0	—	0.048	2.0	++	0.549
3.5	3.0	—	0.113	2.0	++	0.583
4.0	3.2	—	0.099	2.0	++	0.548
5.6	2.8	—	0.187	2.0	++	0.511

1) No visible growth of mycelium.

Table 6

Aspergillus awamori. Duration of culture: 8 days.

Initial pH	No addition of Mn			Addition of Mn (10^{-6} mol)		
	Final pH	Conidium	Dry weight of mycelium (g)	Final pH	Conidium	Dry weight of mycelium (g)
1.2	1.2		0[1]	1.2	—	0.002
1.4	1.4		,,	1.4	—	0.086
1.6	1.6		,,	1.5	—	0.640
1.8	1.8	—	0	1.5	±	0.642
2.2	2.1	—	0.148	1.7	±	0.657
2.8	1.9	—	0.511	1.7	±	0.779
3.6	1.9	—	0.493	1.7	±	0.803
4.2	2.0	—	0.425	1.8	±	0.738
5.5	2.0	—	0.431	1.8	+	0.765

No or poor growth and sporulation of these species of *Aspergillus* in high acidity, which is caused by absence of Mn, is no more the case in the culture to which Mn is added. Mn acts not only to stimulate the growth and sporulation, but also to extend the pH-limit of the fungus growth in high acidity (Photo 50). The same things are experienced in the culture to which Fe (2×10^{-5} mol) or Co (10^{-5} mol)[2] have been added.

Action of Cu upon the formation and the color development of conidium

In the introduction to the present paper it has been mentioned that Cu has an intimate relation with the formation and the color development of conidium of *Aspergillus niger* and *Asp. oryzae.* In the present paper many species of *Aspergillus* were treated in this connection and the results given in the description of the following experiment 5.

Experiment 5.

The culture procedure was almost the same as in the previous experiments. All cultures contained Fe (2×10^{-6}mol), Zn (10^{-6}mol) and Mn (10^{-6}mol). Duration of culture: 6 days.

1) No visible growth of mycelium.
2) The protocols are omitted here.

Table 7

Duration of culture: 6 days.

Fungus	No addition of Cu	Cu (10^{-7} mol)	Cu (10^{-6} mol)	Cu (10^{-5} mol)
	Conidium	Conidium	Conidium	Conidium
Asp. niger	+ ivory buff	++ ivory buff	+++ black	+++ black
Asp. Batatae	+ ivory buff, partially sepia	++ sepia	+++ black	+++ black
Asp. oryzae	± yellow ocher	++ yellow ocher	+++ olive ocher	+++ olive
Asp. flavus	++ Naples yellow	++ Naples yellow	+++ sulphine yellow	+++ chromium green
Asp. parasiticus	++ apricot yellow	+++ pyrite yellow (periphery), orange yellow (centre)	+++ oil green (periphery), orange yellow (centre)	+++ oil green
Asp. tamarii	+++ cream yellow or tawny olive	+++ tawny olive	+++ tawny olive	+++ tawny olive
Asp. Wentii	—	—	—	—
Asp. clavatus	—	++ light porcelain green or artemisia green (periphery), isabella color (centre)	++ light porcelain green or artemisia green	++ light porcelain green or artemisia green

Table 7—(*Continued*)

Fungus	No addition of Cu	Cu (10^{-7} mol)	Cu (10^{-6} mol)	Cu (10^{-5} mol)
	Conidium	Conidium	Conidium	Conidium
Asp. versicolor	± cream yellow	± ocean green (periphery), cream yellow (centre)	+ ocean green	++ ocean green
Asp. japonicus	+ sudan brown	+ sudan brown	+ vitis lake	++ vitis lake or taupe brown
Asp. terreus	—	—	—	—
Asp. ochraceus	± cream yellow	+ cream yellow	+ cream yellow	+ cream yellow
Asp. melleus	+ pale lemon yellow	+++ ivory buff	+++ cream yellow	+++ cream yellow
Asp. awamori	± light brown drab (pale)	± light brown drab (pale)	+ light brown drab	+ light brown drab
Asp. sulphureus	++ apricot yellow	++ apricot yellow	+++ yellow ocher	+++ yellow ocher
Asp. candidus	± white	± white	+ white	+ white
Asp. ustus	± apricot yellow	± apricot yellow	± apricot yellow	+ apricot yellow

Cu benefits the sporulation in general, while in the case of *Asp. clavatus* no conidium is formed in failure to add Cu. However, Cu has sometimes no bearing upon the sporulation, for instance, it does not occur in *Asp. Wentii* and *Asp. terreus*, even if Cu is added. Cu plays an important rôle also in the development of the proper color of conidium, and its omission results in the strange appearance of the fungus felt in respect to the color. The minimum concentration of Cu, in which its action appears, is 10^{-6}mol. The more concentrated the Cu is, the deeper appears the proper color of the conidium.

Cd affects the formation and color development of conidia, rather acting against the development of the proper color of conidium. The following experiment 6 treats of this relation.

Experiment 6.

The cultures was carried out similarly to the procedure in the foregoing experiments. Cu (10^{-6}mol and Mn (10^{-6}mol) were previously added to the standard solution. Duration of culture: 7 days.

Table 8

Fungus	No addition of Cd		Addition of Cd (10^{-5} mol)	
	Sporulation	Color	Sporulation	Color
Asp. niger	+++	black	++	ecru or maple
Asp. oryzae	+++	olive ocher	++	Naples yellow
Asp. flavus	++	sulphine yellow	++	apricot yellow
Asp. parasiticus	++	olive green (periphery), orange yellow (centre)	+	pyrite yellow or sulphine-yellow
Asp. clavatus	+++	light porcelain green or artemisia green	−	white

Parallel to the above experiment some other ones were carried out, where the action of Ni, Co and Al on the sporulation and the color development of conidia was examined, but no remarkable effects could be seen. The details are, therefore, omitted here.

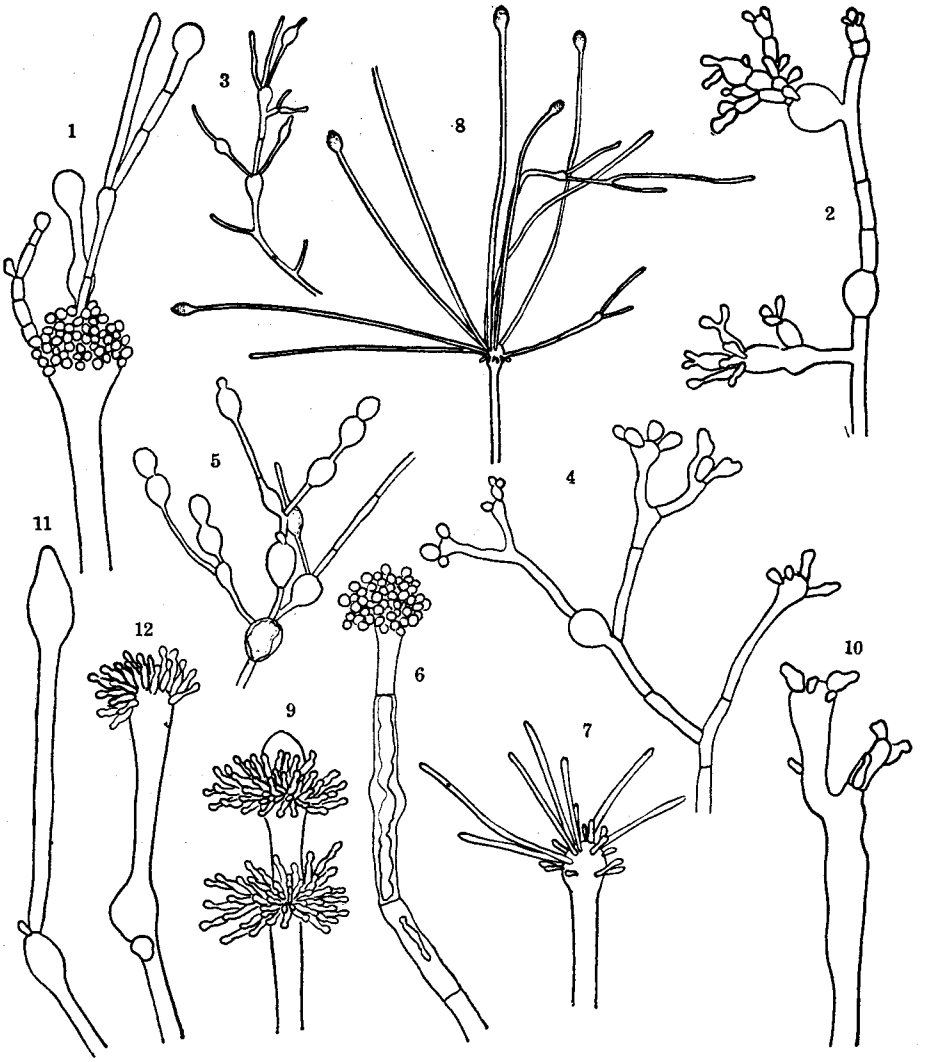

Fig. 1–12. *Aspergillus oryzae.*
1–6. Cu (10^{-6}mol).
7. Mn (10^{-5}mol).
8. Mn (10^{-4}mol).
9. Mn (10^{-5}mol).
10–12. Mn (10^{-6}mol) + Cu (10^{-6}mol) + Cd (10^{-4}mol).

Magnifications:
Fig. 8 × 37.
Fig. 3. × 85.
Fig. 5, 11, 12, 14. × 139.
Fig. 1, 4, 6, 7, 9, 10. × 216.
Fig. 2. × 328.

Fig. 13 and 14, *Aspergillus oryzae.*
13, 14, Mn (10^{-6}mol) + Cu (10^{-6}mol) + Cd (10^{-4}mol).
Fig. 15–25. *Aspergillus flavus.*
15–23. Cu (10^{-6}mol).
24, 25. Mn (5×10^{-8}mol) + Cu (10^{-6}mol).

Magnifications:
Fig. 14. × 139.
Fig. 13, 23–25. × 216.
Fig. 15–22. × 328.

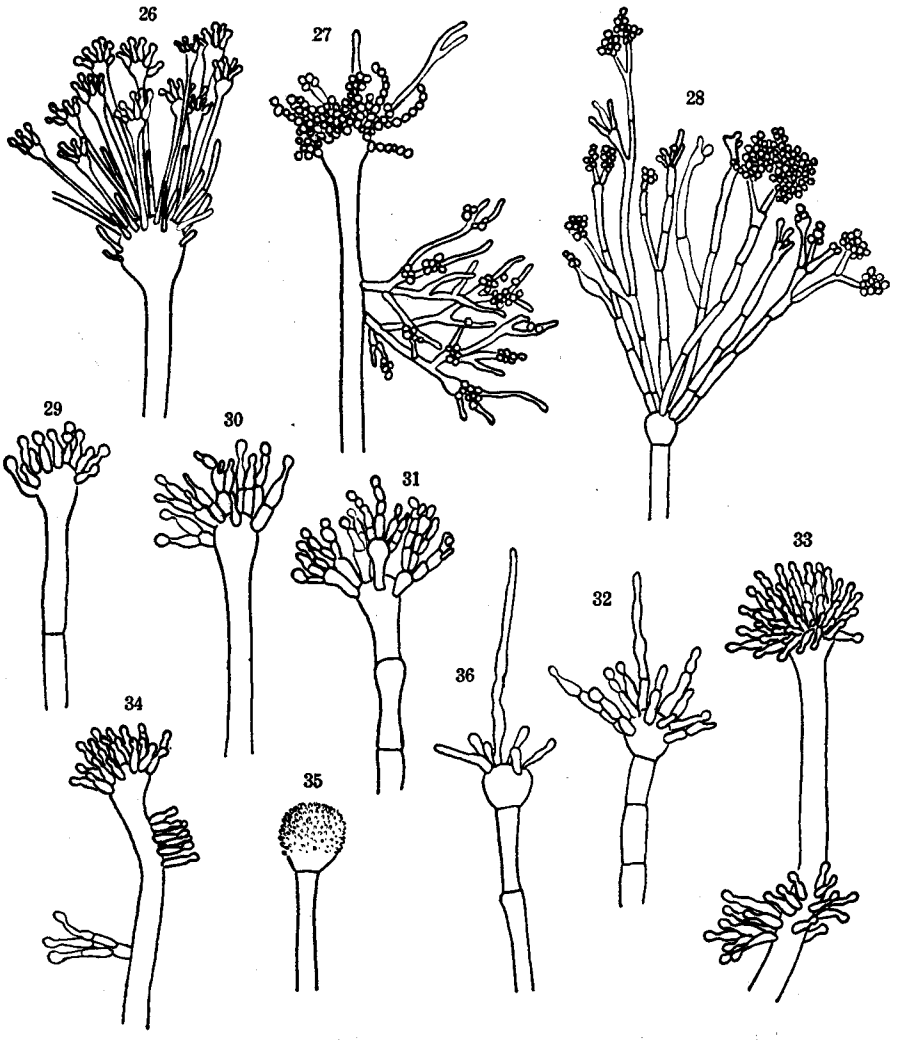

Fig. 26–36. *Aspergillus flavus.*
26–28. Mn (5×10^{-8}mol) + Cu (10^{-6}mol).
29. Mn (10^{-6}mol).
30–32.[1] Mn 10^{-6}mol).
33, 34. Mn (10^{-4}mol).
35, 36. Mn (5×10^{-8}mol).

Magnifications:
Fig. 26–28,35,36. × 216.
Fig. 29–34. × 328.

1) Zn (10^{-5}mol).

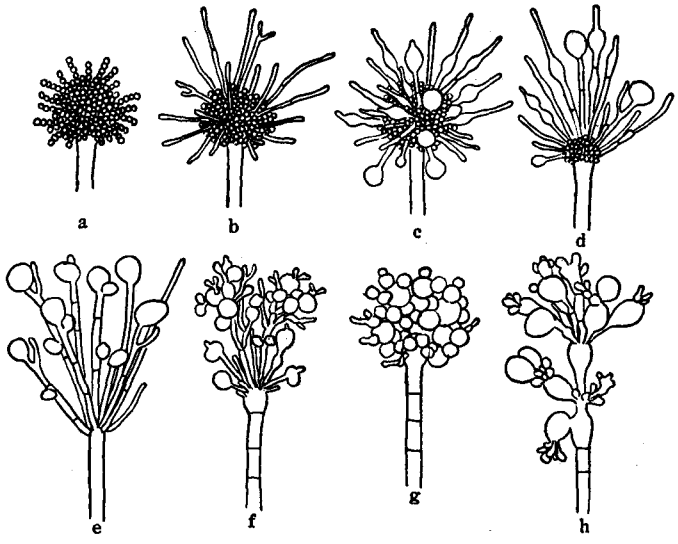

Fig. 37. A diagrammatic illustration of transition forms from the normal conidium to the spherical cell in *Asp. oryzae*.

Although it is now beyond doubt that Cu has a very important rôle on the sporulation, other factors must not be ignored in this relation. It has been seen in experiment 5 that in *Asp. Wentii* and *Asp. terreus* the sporulation never occurs, even if sufficient Cu was added. From the experience of the ordinary culture of *Aspergilli* the present writer has learned that the diminution of the quantity of sugar results in easy promotion of the conidium formation. This tendency was confirmed by an exact culture experiment, of which the result is reported below.

Experiment 7.

The quantity of glucose was reduced to 1/10. Other cultural conditions were the same as in the previous experiments. To the standard solution were added Cu (10^{-6}mol) and Mn (10^{-6}mol). Duration of culture: 6 days.

Table 9

Fungus	Sporulation	
	Reduced sugar quantity	Non-reduced sugar quantity
Asp. Wentii	+	−
Asp. terreus	++	−
Asp. awamori	+++	+
Asp. ustus	+	±

It is possible by this means to induce the sporulation in such fungi as *Asp. Wentii* and *Asp. terreus,* where it hardly takes place in the ordinary culture.

Summary

1. In many species of *Aspergillus* the sporulation is favored by Cu, but its action is perfected by the addition of Mn. For the development of fungus cells in the mycelial form, if Cu is present, Mn is an indispensable element. The absence of Mn causes the spherical cell formation.

2. The proper combination of Cu (or Zn) and Mn gives rise to the formation of the normal conidia-bearing head. Omission of either one of the two causes the reduction of the sporulation and the abnormal development of the conidial head.

3. Transition stages from the conidial formation to the spherical cell formation can be brought about experimentally when the quantity of Mn is varied. It is, therefore, very probable that an intimate relation exists between these two tendencies of the cell modification.

4. Mn acts extending the pH-limit of the fungus growth in high acidity.

5. Cu plays an important rôle for the development of the proper color of the conidium of Aspergilli. The minimum concentration of Cu, in which it clearly influences the sporulation and the color development of the conidium is 10^{-6}mol.

6. Diminution of the sugar quantity also favors the sporulation.

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Explanation of Plates

To all culture solutions were added Fe (2×10^{-6}mol) and Zu (10^{-6}mol) except in a few cases. The further addition of Mn and Cu is here indicated.

Plate IV

Photo 1–3. *Aspergillus niger.*
1, 2. Mn (10^{-6}mol).
3. Cu (5×10^{-3}mol).

Photo 4–9. *Aspergillus oryzae.*
4. Mn (10^{-6}mol) + Cu (10^{-6}mol). Normal head.
5. Mn (10^{-8}mol) + Cu (10^{-6}mol).
6. Cu (10^{-6}mol).
7. Cu (10^{-7}mol).
8. Mn (5×10^{-8}mol) + Cu (10^{-6}mol).
9. Mn (10^{-8}mol) + Cu (10^{-6}mol).

Magnifications:

Fig. 3. × 48.
Fig. 1, 5, 6. × 125.
Fig. 2, 9. × 195.
Fig. 4, 7. × 250.
Fig. 8. × 377.

Plate V

Photo 10–19. *Aspergillus oryzae.*
10. Mn (5×10^{-8}mol) + Cu (10^{-6}mol).
11, 12. Cu (2×10^{-6}mol).
13. Mn (5×10^{-8}mol) + Cu (10^{-6}mol).
14. Mn (10^{-6}mol).
15. Mn (10^{-5}mol).
16. Mn (10^{-4}mol).
17. Mn (10^{-6}mol).
18. Mn (10^{-5}mol).
19. Mn (5×10^{-8}mol) + Cu (10^{-6}mol).

Magnifications:

Fig. 18. × 48.
Fig. 13, 19. × 125.
Fig. 10, 16. × 195.
Fig. 11, 14, 15, 17. × 250.
Fig. 12. × 377.

Plate VI

Photo 20–23. *Aspergillus oryzae.*
20–23. Mn (10^{-6}mol) + Cu (10^{-6}) + Cd (10^{-4}mol).
Photo 24–29. *Aspergillus flavus.*
24. Mn (10^{-6}mol) + Cu (10^{-6}mol). Normal head.
25–28. Cu (10^{-6}mol).
29. Mn (5×10^{-8}mol) + Cu (10^{-6}mol).

Magnifications:

Fig. 23. × 125.
Fig. 21. × 195.
Fig. 20. 22–26, 27, 29. ×250.
Fig. 27. ×377.

Plate VII

Photo 30–39. *Aspergillus flavus.*
30–36.[1] Mn (5×10^{-8}mol) + Cu (10^{-6}mol).
37, 38. Mn (5×10^{-8}mol) + Cu (10^{-7}mol).
39. Mn (5×10^{-8}mol) + Cu (5×10^{-8}mol).

Magnifications:

Fig. 32. × 125.
Fig. 35. × 195.
Fig. 30, 31, 33, 34, 37–39. × 250.
Fig. 36. × 508.

Plate VIII

Photo 40–44. *Aspergillus flavus.*
40. Mn (10^{-6}mol).
41, 42.[2] Mn (10^{-6}mol).
43. Mn (10^{-4}mol).
44. Mn (10^{-5}mol).
Photo 45. *Aspergillus candidus.*
45. Mn (10^{-6}mol).
Photo 46. *Aspergillus parasiticus.*
46. Mn (10^{-6}mol).
Photo 47. *Aspergillus tamarii.*
47. Cu (10^{-6}mol).
Photo 48, 49. *Aspergillus flavus.*
48, 49. Mn (10^{-6}mol) + (Cu (10^{-6}mol). Temperature: 40°C.

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1) In Photo 35 and 36, long culture duration.
2) Zn (10^{-5}mol).

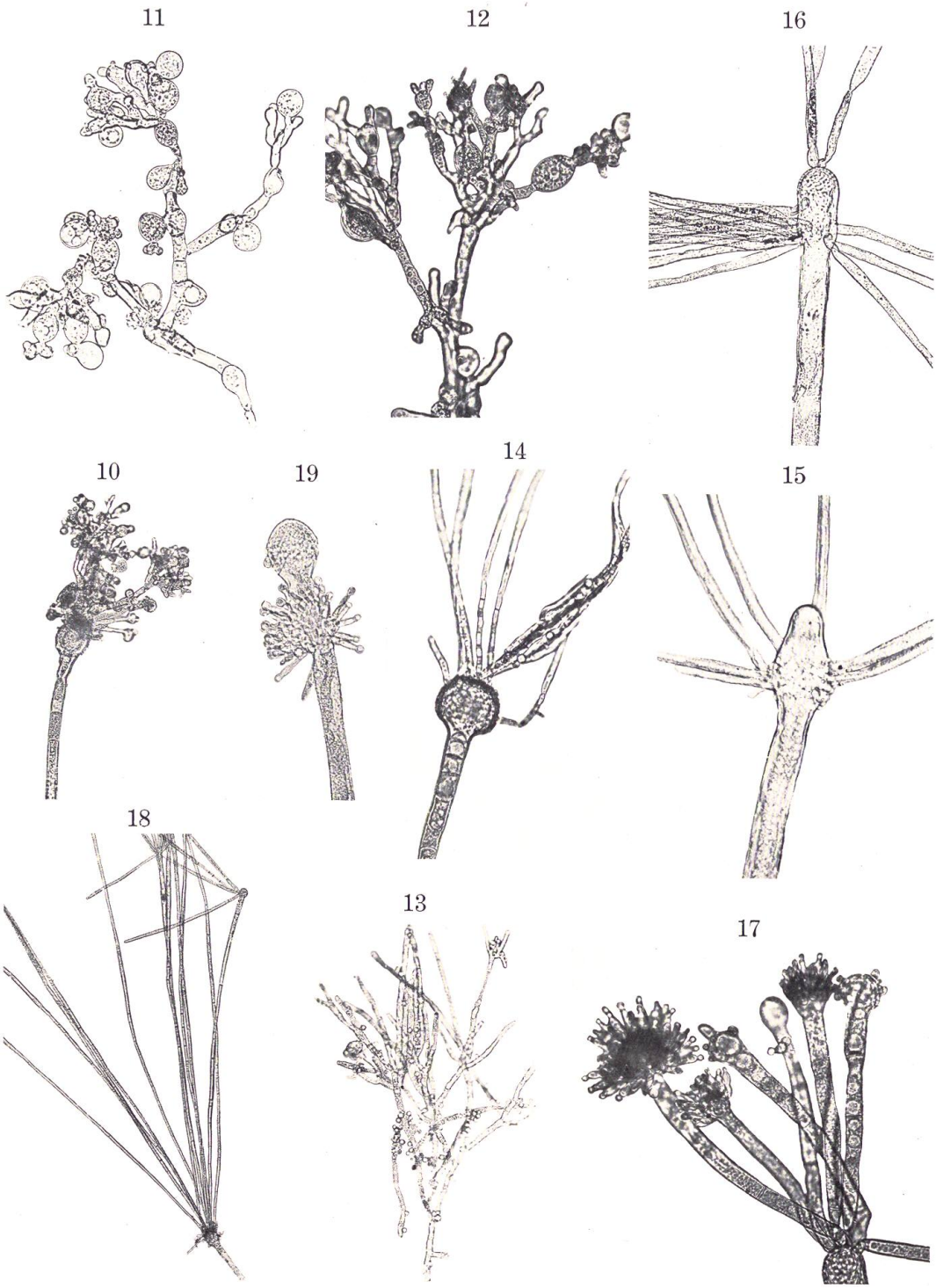
Magnifications:

Fig. 45.	× 125.
Fig. 46.	× 185.
Fig. 47.	× 195.
Fig. 43.	× 250.
Fig. 48, 49.	× 278.
Fig. 40–42, 44.	× 377.

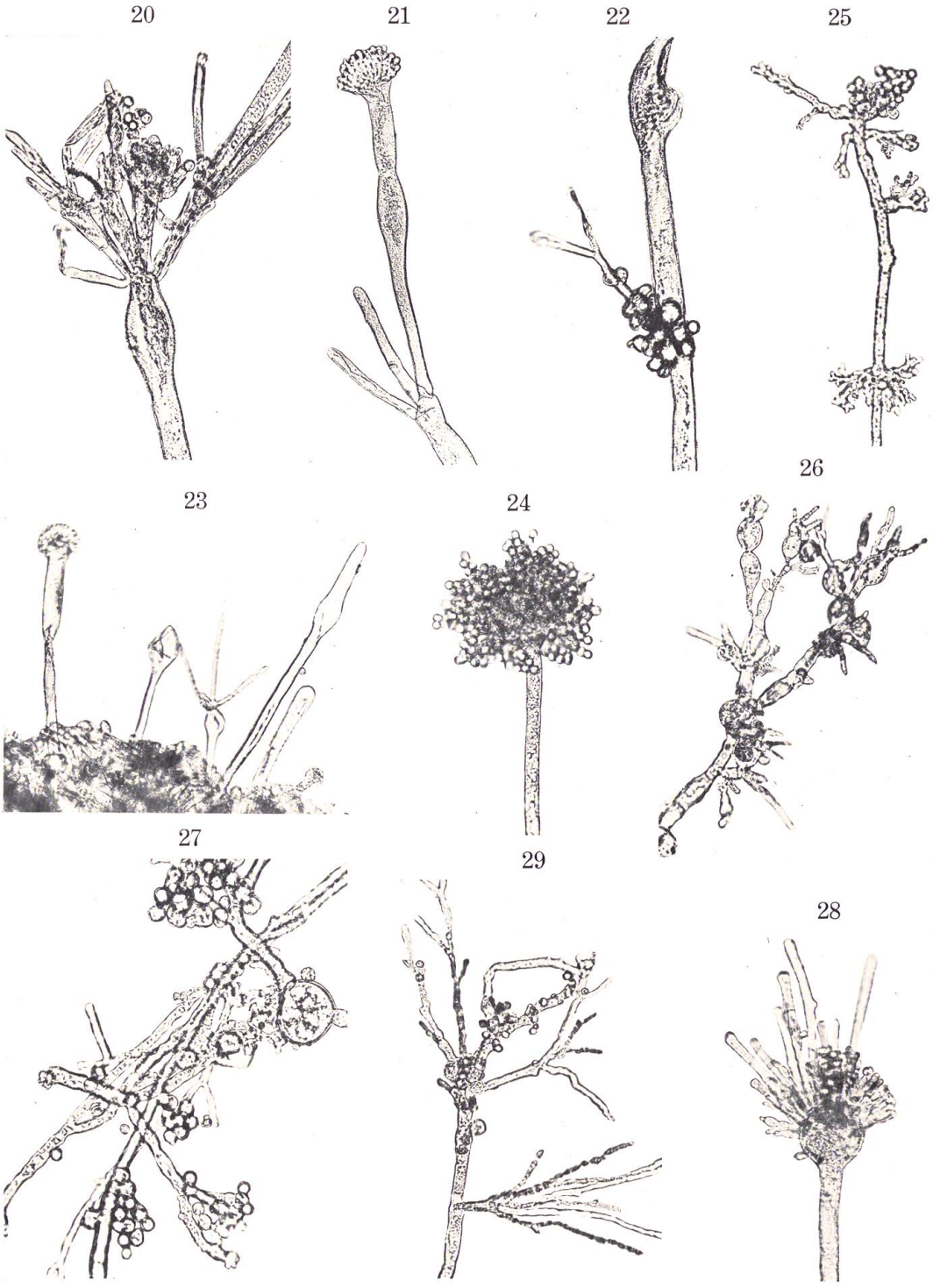
Plate IX

Photo 50. *Aspergillus niger.*
Series A, Fe (2×10^{-6}mol) + Zn (10^{-6}mol) + Cu (10^{-6}mol).
Series B, Fe (2×10^{-6}mol) + Zn (10^{-6}mol) + Cu (10^{-6}mol). + Mn (10−6mol).

Yoshimura: Action of Copper and Manganese.



Yoshimura: Action of Copper and Manganese.



Yoshimura : Action of Copper and Manganese.

Yoshimura: Action of Copper and Manganese.

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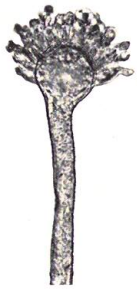

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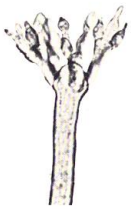

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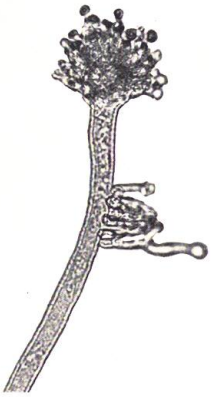

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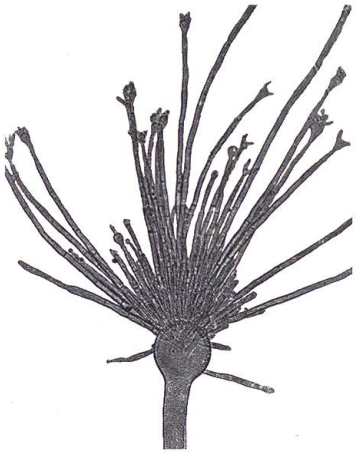

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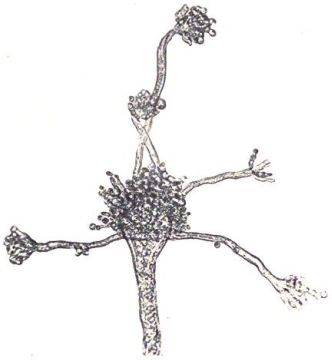

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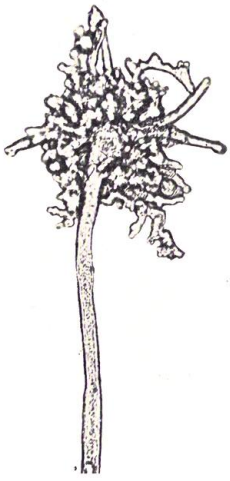

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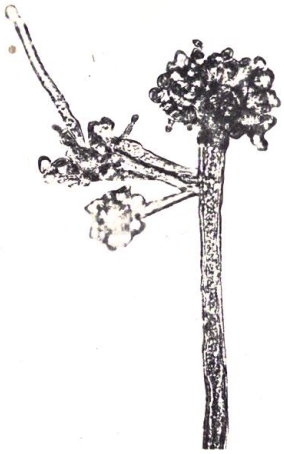
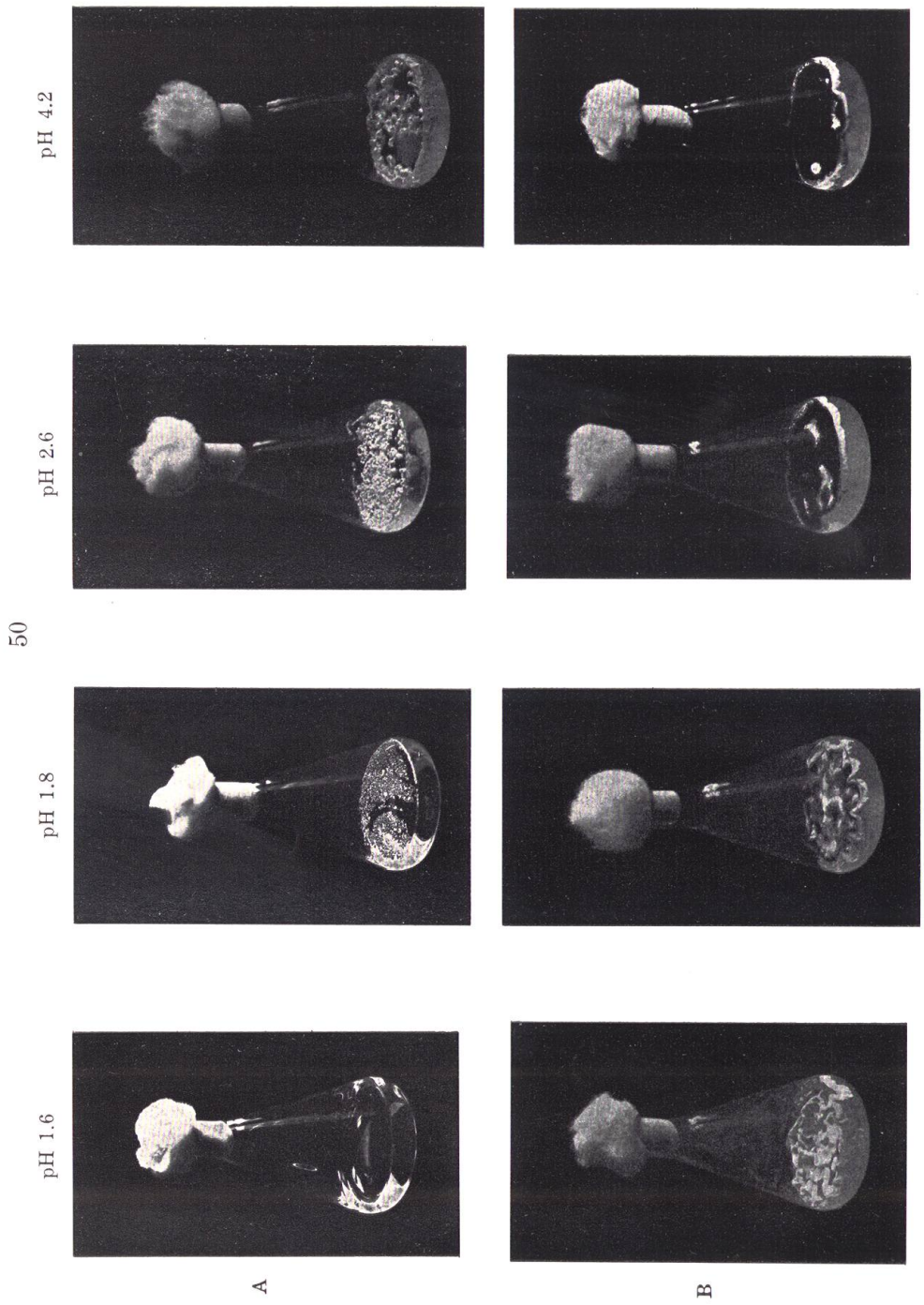


Yoshimura : Action of Copper and Manganese.



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Yoshimura : Action of Copper and Manganese.