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Title	Studies on the Effects of Fat-soluble Vitamin upon the Growth of some Parasitic Fungi
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Citation	Journal of the Faculty of Agriculture, Hokkaido Imperial University, 32(3), 71-107
Issue Date	1933-06-28
Doc URL	https://hdl.handle.net/2115/12687
Type	departmental bulletin paper
File Information	32(3)_p71-107.pdf

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STUDIES ON THE EFFECTS OF FAT-SOLUBLE VITAMIN UPON THE GROWTH OF SOME PARASITIC FUNGI

By

Yoshihiko Tochinai and Mutsuo Terui

(With 2 plates and 16 text figures)

Introduction

Since the vitamin-theory was established by FUNK (1911) in the nutrition of animals, studies on the influences of vitamin B upon the growth of microorganisms have been carried out by several authors. Most authors have used yeasts and fungi which are concerned in agricultural technology. They dealt with water-soluble vitamin B in their studies. Several investigators have reported upon the favorable influence of vitamin B on the development of yeast or other groups of fungi, but as a whole it seems that the effects of vitamin B on the heterotrophic fungi are other than the general conception of the influence of vitamin in the nutrition physiology of animals. Recently S. ITO and M. TERUI having studied the influence of oryzanin (vitamin B) on the development of *Ophiobolus Miyabeanus* ITO et KURIBAYASHI, *Gibberella Fujikuroi* (SAWADA) ITO, and *Piricularia Oryzae* BR. et CAV., reported that the addition of oryzanin to the synthetic culture media has a certain favorable effect on the germination of conidia, formation of conidia and the development of mycelia. They have examined vitamin B not only as a stimulating also as a nutrient substance, and found that the vitamin B having been quite efficaciously utilized as carbon- or nitrogen-sourse for the nutrition of the fungi, plays also an important rôle as an ordinary nutritive substance rather than as a special growth-promoting principle.

[Jour. Facul. Agr., Hokkaido Imp. Univ., Sapporo, Vol. XXXII, Pt. 3, March, 1932.]

In actual fact only a few studies of the influence of vitamins on plant pathogenic fungi have been carried out. Out of the studies done recently by ITO and TERUI, WILLAMAN (1919) reported the necessity of adding vitamin B for the development of *Sclerotinia cinerea* parasitic on the fruits of *Prunus Mume* and *Prunus Persica* in culture medium containing no other organic substances than asparagine and sucrose. In 1928 YAMAMOTO reported similar data to WILLAMAN'S on *Sclerotinia Mali* parasitic on apples in his graduation thesis deposited in the Faculty of Agriculture, Hokkaido Imperial University.

Those studies have been done on vitamin B, but as far as the authors know no studies of the influence of vitamin A on the nutrition of fungi have been reported up to the present time.

Vitamin A is known as a fat-soluble principle being necessary for the normal growth of animals, but its physical and chemical properties are still more unknown than those of vitamin B.

The junior author having studied jointly with Prof. S. ITO on vitamin B about the development of several fungi in artificial culture media, assumed its undoubtedly profitable nutritive effect as the carbon- or nitrogen-source and also some occasional stimulating effects as an accessory substance. Under these circumstances the authors' concern turned to fat-soluble vitamin as a matter of course.

The Methods and Materials

As the sources of the vitamin "Riken vitamin A" and "Biosterin" which are prepared and sold by the Institute of Physical and Chemical Research of the Japanese Government were used.

"Riken vitamin A" is an about 1.2% olive oil solution of so-called vitamin A extracted from cod-liver oil by the process of eliminating saponified substances from cod-liver oil and then from that residue, the unsaponified substances, eliminating cholesterin (TAKAHASHI, K. and K. KAWAKAMI, 1923).

"Biosterin" is also a 1.5–2% olive oil solution of vitamin A prepared for use in injection. The name "Biosterin" was adopted by the late Dr. K. TAKAHASHI who studied vitamin A and assumed it to be a kind of sterol.

MCCOLLUM and co-workers (1925), however, distinguished vitamin D from vitamin A according to the functional differences. Considering the methods of preparation of vitamin A used in the Institute of Physical and Chemical Research either "Riken vitamin A" or "Biosterin"

probably contains the so-called vitamin D to some extent.

Under the present status of studies on vitamins, the conceptions of every vitamin being unceasingly advancing and varying, the authors used their materials as the sources of vitamin A in the conception hitherto current, understanding that they contain probably the so-called vitamin D other than vitamin A.

As fungus materials for the examinations the following four kinds of important plant pathogenes were used:

1. *Helminthosporium turcicum* PASS. parasitic on *Zea Mais,* collected and supplied by the Agricultural Experiment Station of the Hokkaido Government, and isolated by the junior author.

2. *Ophiobolus Miyabeanus* ITO et KURIBAYASHI (=*Helminthosporium Oryzae*) parasitic on *Oryza sativa,* isolated from affected rice grains by the authors.

3. *Gibberella Fujikuroi* (SAWADA) ITO parasitic on *Oryza sativa* and producing so-called "Bakanae" disease, isolated from affected rice straws by the authors.

4. *Glomerella Lindemuthiana* (SACC. et MAG.) SHEAR (=*Colletotrichum Lindemuthianum*) parasitic on *Phaseolus* species, isolated and supplied by the courtesy of the Agricultural Experiment Station of the Hokkaido Government.

The cultures of the fungus materials cited above were started by single spores and the experiments were carried out in their conidial stages.

For the culture medium of the experiments RICHARDS' synthetic solution was used with an addition of 1.5% agar. To this culture medium each of the vitamin A sources, "Riken vitamin A" or "Biosterin," was added in the proportions of 10%, 5%, 1%, 0.5%, 0.1%, 0.05% and 0.01%. With an addition of control cultures without vitamin A a series of 8 kinds of plate cultures differing in the contents of vitamin A was thus prepared in triplicate for each fungus and each source of vitamin A. Culture plates were prepared in PETRI-dishes having 9 c.m. diameter with 15 c.c. of each of the culture media, and sterilized three times in a KOCH'S steam sterilizer for 30 minutes on three successive days. The stability of vitamin A in relation to sterilization will be discussed in the following chapter.

The culture-plate was inoculated at the center with a bit of each fungus, and incubated in a thermostat at 26°–28°C. The increase in the diameter of the fungus colonies was measured every day until the

best growth occupied the entire surface of the culture medium. At the same time the appearance of the fungus colonies was observed. In the cases of *Glomerella Lindemuthiana,* however, the development of the hyphal colonies was exceptionally slow, and the measurement of their diameter was made every other day.

The pharmacons of the vitamin A used in the present studies being prepared as the olive oil solutions, to find out the influence of olive oil itself should be of great importance. Having this in mind a series of olive oil cultures was carried out parallel to the vitamin A cultures under the similar conditions.

Thermo-Stability of Vitamin A

Preliminarily to the preparation of the culture media containing vitamin A, tests of its thermo-stability have been carried out.

In spite of the lack in accurate knowledge of the physical and chemical properties of vitamin A, it is generally believed to be rather stable to heating. This has been induced from several investigations published up to the present time. OSBORNE and MENDEL (1915) reported that the growth-promoting action of butter was not destroyed by a treatment of steam for 2 hours and 30 minutes. HOPKINS (1920) reported that vitamin A contained in butter remained almost unchanged through a treatment in an autoclave at 120°C. for 4 hours, but it was largely destroyed by heating in the air at the same temperature for an equal duration, and even at 80°C. a comparatively easy destruction was experienced. STEENBOCK and BOUTWELL (1920) reported that vitamin A contained in carrots and pumpkins remained completely undestroyed through an autoclaving at 120°C. for 3 hours. Out of the reports cited above, DRUMMOND (1919) who studied the nature and properties of vitamin A, DRUMMOND and COWARD (1920) who studied the effect of heat and oxygen on the nutritive value of butter, POWICK (1925) who studied the destruction of vitamin A according to the rancidity of fat, DRUMMOND, CHANNON and COWARD (1926) who made chemical studies of vitamin A, and SHERMAN, QUINN, DAY and MILLER (1928) who studied the relative stability of vitamin A extracted from plant materials, have reported also that vitamin A is fairly stable to high temperature treatments such as 120°C.

It would be induced from the results reported in these former investigations that vitamin A is evidently stable against ordinary high temperature when it is protected from severe oxidation. Accordingly

it is safely inferred that the sterilization of the media containing vitamin A in KOCH'S steam sterilizer will only slightly affect its stability. It should be of consequence, however, to prove this conjecture empirically. With this intention examination was made as to whether or not the different temperatures used for the sterilization of culture media containing vitamin A causes any difference in the development of a fungus.

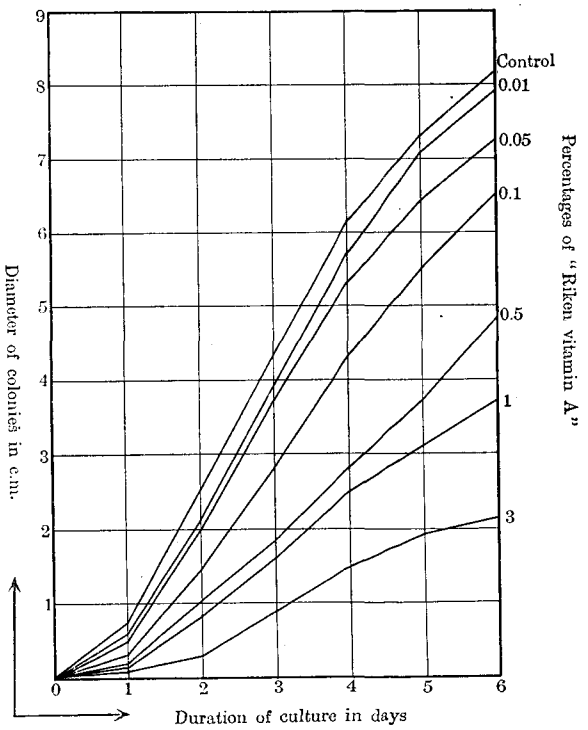
TABLE I.—*Average diameter in c.m. of the colonies of Helminthosporium turcicum developed on media containing "Riken vitamin A" in various percentages and sterilized higher or lower temperature*

Sterilization	Content of Riken vitamin A \ Duration of culture in days	1	2	3	4	5	6
Higher temperature	Control	0.76	2.50	4.34	6.12	7.34	8.16
	0.01 %	0.60	2.10	3.90	5.66	7.10	7.98
	0.05 %	0.56	2.00	3.70	5.30	6.44	7.26
	0.1 %	0.30	1.42	2.80	4.32	5.52	6.56
	0.5 %	0.22	1.04	1.82	2.82	3.72	4.94
	1 %	0.20	0.88	1.60	2.48	3.10	3.70
	3 %	0.14	0.34	0.90	1.46	1.92	2.14
Lower temperature	Control	0.66	1.96	3.74	5.54	6.88	8.10
	0.01 %	0.74	1.96	3.72	5.50	6.72	7.84
	0.05 %	0.63	1.84	3.46	5.30	6.36	7.40
	0.1 %	0.30	1.54	2.92	4.40	5.30	5.96
	0.5 %	0.24	0.98	1.78	2.70	3.36	4.14
	1 %	0.20	0.64	1.46	2.34	3.00	3.66
	3 %	0.10	0.33	0.63	1.06	1.50	2.00

Two series of culture media containing vitamin A in the proportions of 3%, 1%, 0.5%, 0.1%, 0.05% and 0.01% were prepared. Each medium of each series was divided among five PETRI-dishes as culture-plates. A series of the culture-plates was sterilized in a KOCH'S steam sterilizer at 100°C. for 30 minutes each on three successive days. The other series was sterilized in a thermo-regulating water bath at 68°–70°C. for one hour each on three successive days and then twice more at one day intervals. *Helminthosporium turcicum* was inoculated at the cen-

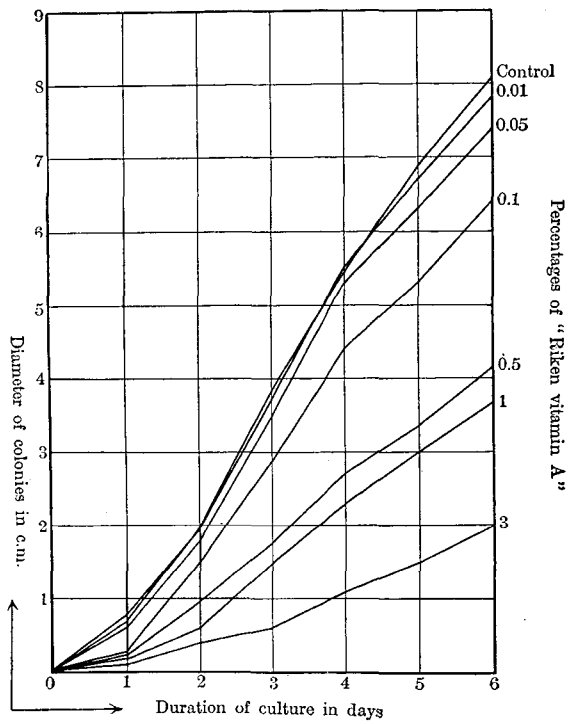
tres of the culture-plates. The culture work was carried out and the cultural data were observed according to the schemes described in the preceding chapter. The diameter of the colonies developed on the plate-cultures has been measured in c.m. every day until 6 days after inoculation. The data are shown in table I and figures I–III.

Fig. I. The growth of *Helminthosporium turcicum* on culture media containing "Riken vitamin A" in various percentages and sterilized at higher temperature (100°C.)



The difference in growth-rate observed between these two series of cultures was almost negligible at first sight, but the diameters of the fungus colonies developed on the culture media sterilized at higher temperature for shorter duration measured in general slightly larger than those of the other series of cultures. The appearance of the fungus growth also showed no marked difference due to the difference in the methods of sterilization of the media. In either series of cultures the

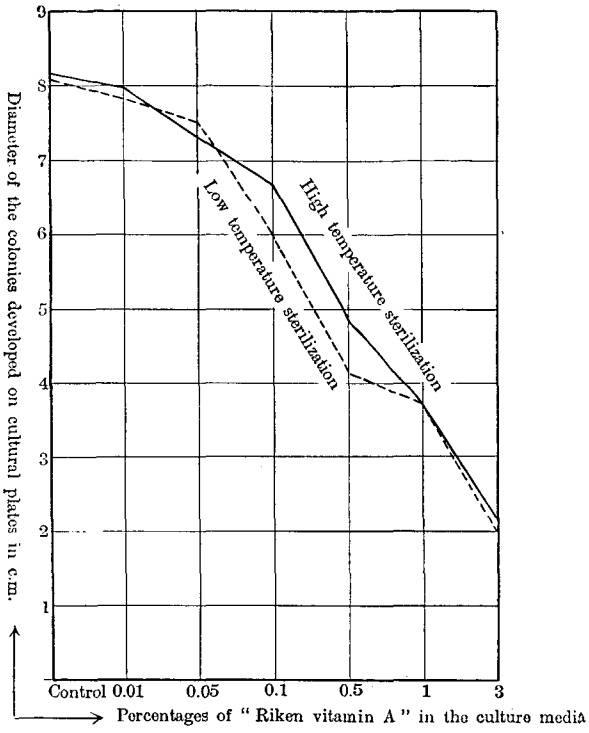
Fig. II. The growth of *Helminthosporium turcicum* on culture media containing "Riken vitamin A" in various percentages and sterilized at lower temperature (68°–70°C.).



hyphal developments of *Helminthosporium turcicum* were markedly retarded by the increase of content of the vitamin A in the culture media, and a similar effect was observed also in the formation of conidia.

It may be properly assumed from the results obtained in the present experiments that the sterilization of the culture media in KOCH's steam sterilizer at 100°C. for 30 minutes thrice on successive three days has no worse effect upon the stability of vitamin A than the sterilization at lower temperature with accordingly necessary longer duration. This conclusion seems to coincide essentially with the results of the former investigations of the thermo-stability of vitamin A, cited above. The proof of such thermo-stable character of vitamin A made it very convenient in the further investigations on the present line to adopt the most common method of sterilization in preparation of culture media containing vitamin A.

Fig. III. Curves comparing the growth of *Helminthosporium turcicum* developed in 6 days on culture media containing "Riken vitamin A" in various percentages and sterilized at higher or lower temperature.



Experiments on the Influences of the Vitamin A upon the Growth of *Helminthosporium turcicum*

In the present experiments three series of cultures were studied in parallel. The fungus was cultured on media containing pure olive oil in the first series, "Riken vitamin A" in the second series, and "Biosterin" in the third series, the content ranging from 10% to 0.01%. Cultures on the standard culture medium containing no vitamin A or olive oil were taken as the controls in every series.

The developments of the fungus colonies on every plate-culture were observed and measured every day until 6 days passed from the inoculation. The average diameters of the colonies measured daily on each of the three cultures are shown in tables II, III, and IV and figures

IV, V, and VI. The differences of hyphal developments due to the varying percentages of olive oil, "Riken vitamin A", and "Biosterin" were measured at the end of the culture period and graphed in Figure VII.

TABLE II.—*Average diameter in c.m. of colonies of Helminthosporium turcicum developed on media containing olive oil in various percentages*

(a) First Experiment

Content of olive oil \ Duration of culture in days	1	2	3	4	5	6
Control	0.94	1.95	4.03	5.75	7.35	8.53
0.01 %	0.85	1.85	3.63	5.36	7.06	8.33
0.05 %	0.70	1.80	3.73	5.50	7.06	8.43
0.1 %	0.70	1.73	3.53	5.26	7.10	8.26
0.5 %	0.56	1.70	3.64	5.56	7.56	8.56
1 %	0.63	1.80	3.56	5.76	7.30	8.66
5 %	0.66	1.76	3.70	5.86	7.60	8.63
10 %	0.54	1.63	3.86	5.90	7.46	8.80

(b) Second Experiment

Content of olive oil \ Duration of culture in days	1	2	3	4	5
Control	1.93	3.53	5.46	7.06	8.70
0.01 %	2.10	3.63	5.73	7.00	8.50
0.05 %	1.80	3.63	5.56	7.23	8.70
0.1 %	1.80	3.80	5.80	7.40	8.73
0.5 %	2.03	3.83	5.90	7.66	8.73
1 %	1.80	3.76	5.90	7.53	8.70
5 %	1.46	3.13	5.50	7.46	8.70
10 %	1.56	3.46	5.50	7.53	8.80

TABLE III.—*Average diameter in c.m. of colonies of Helminthosporium turcicum developed on media containing "Riken vitamin A" in various percentages*

(a) First Experiment

Content of Riken vitamin A \ Duration of culture in days	1	2	3	4	5	6
Control	1.13	2.40	4.10	6.06	7.46	8.50
0.01 %	1.06	2.30	3.70	5.66	7.23	8.30
0.05 %	0.96	2.13	3.58	5.35	7.10	8.26
0.1 %	0.90	2.00	3.43	5.03	6.36	7.40
0.5 %	0.90	1.75	3.00	4.30	5.35	6.25
1 %	0.80	1.60	2.55	3.65	4.35	5.20
5 %	0.73	1.26	1.90	2.23	2.66	3.06
10 %	0.50	0.83	1.20	1.53	1.70	1.83

(b) Second Experiment

Content of Riken vitamin A \ Duration of culture in days	1	2	3	4	5	6
Control	1.23	2.46	4.03	5.73	7.10	8.33
0.01 %	1.20	2.23	3.60	5.26	6.73	8.13
0.05 %	0.96	2.03	3.46	5.16	6.40	7.70
0.1 %	0.93	1.96	3.36	4.80	6.10	7.13
0.5 %	0.90	1.86	3.16	4.40	5.53	6.23
1 %	0.86	1.76	2.96	4.26	5.40	6.23
5 %	0.86	1.53	2.20	2.86	3.33	3.73
10 %	0.56	1.13	1.66	2.10	2.33	2.56

In these cultural studies of *Helminthosporium turcicum,* olive oil contained in the culture medium had no special apparent effect upon the growth of the fungus. The daily increase in diameter of the fungus colonies on every culture varying in the content of olive oil was almost equal in all cases. The growth rates, therefore, were traced almost parallel as shown in the figure IV. The aerial mycelium grew vigorously in every case showing pallid neutral gray, while the submerged mycelium developed in the culture medium appeared brownish drab

TABLE IV.—*Average diameter in c.m. of colonies of Helminthosporium turcicum developed on culture media containing "Biosterin" in various percentages*

(a) First Experiment

Content of Biosterin \ Duration of culture in days	1	2	3	4	5	6
Control	1.16	2.50	4.10	5.73	7.56	8.46
0.01 %	1.00	2.26	3.90	5.70	7.46	8.43
0.05 %	0.93	2.06	3.56	5.36	7.23	8.36
0.1 %	0.83	1.93	3.33	5.16	6.93	8.06
0.5 %	0.73	1.66	3.03	4.63	6.26	7.56
1 %	0.80	1.63	2.76	3.90	5.26	6.63
5 %	0.76	1.46	2.43	3.33	4.40	5.43
10 %	0.63	1.36	2.03	2.76	3.53	4.26

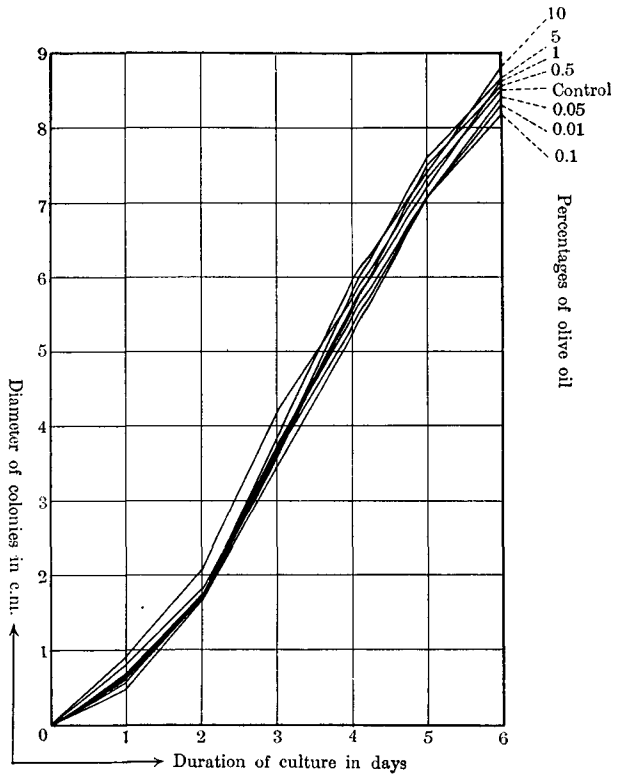
(b) Second Experiment

Content of Biosterin \ Duration of culture in days	1	2	3	4	5
Control	1.90	3.23	5.28	6.92	8.10
0.01 %	1.56	3.30	5.36	6.93	8.03
0.05 %	1.66	3.40	5.20	6.90	7.70
0.1 %	1.56	3.16	4.70	6.40	7.35
0.5 %	1.50	3.03	4.43	6.05	7.25
1 %	1.43	2.53	3.76	5.40	6.80
5 %	1.66	2.63	3.63	5.06	6.13
10 %	1.36	2.33	3.16	4.70	5.76

or fuscous black in color. The formation of conidia was observed only slightly in every case within the present duration of culture. From the results obtained from these cultural studies it is concluded that olive oil, which is used generally as the pharmaceutical solvent of vitamin A, has almost no effect upon the growth of *Helminthosporium turcicum*.

In the cases of the cultures containing "Riken vitamin A" and

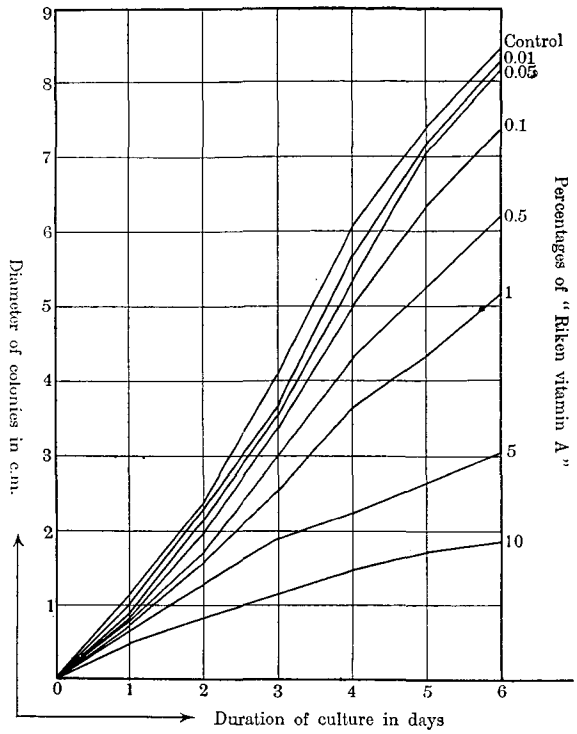
Fig. IV. The growth of *Helminthosporium turcicum* on culture media containing olive oil in various percentages. (Data obtained in the first experiment.)



"Biosterin", however, extremely interesting results have been observed. The growth of the fungus on the culture media containing vitamin A was observed to be evidently inferior to that on the control cultures, and the declination of the hyphal development was intensified remarkably by the increase of the content of either "Riken vitamin A" or "Biosterin" in the culture media. Aerial mycelium showed pallied neutral gray and submerged mycelium showed fuscous black in color on every culture. Only a slight formation of conidia was observed.

As the results obtained in the present three series of culture experiments concerning olive oil, "Riken vitamin A" and "Biosterin" seem to be somewhat unexpected, just similar culture experiments were repeated twice more. The data obtaind in those proving experiments confirmed the foregoing results exactly.

Fig. V. The growth of *Helminthosporium turcicum* on culture media containing "Riken vitamin A" in various percentages. (Data obtained in the first experiment.)

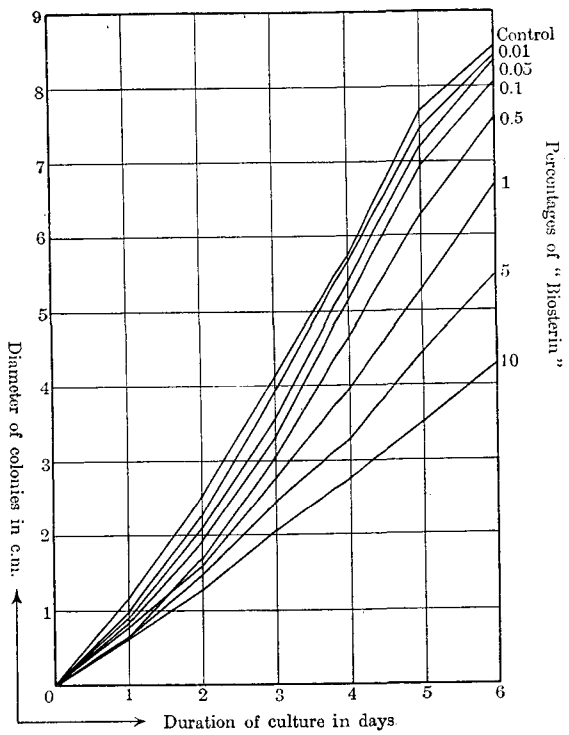


Considering these results synthetically it is sure to be concluded that vitamin A behaves as a growth-retarding principle on *Helminthosporium turcicum* at least under the present cultural conditions.

Experiments on the Influence of the Vitamin A upon the Growth of *Ophiobolus Miyabeanus*

A marked retardation of hyphal growth resulting from the presence of vitamin A in culture media having been observed in the foregoing experiments with *Helminthosporium turcicum,* similar culture experiments were carried out on another species of the same genus, *Ophiobolus Miyabeanus.* This had been known formerly as *Helminthosporium Oryzae,* but the perfect stage of the fungus having been found, the present name was given by Ito and Kuribayashi (1927).

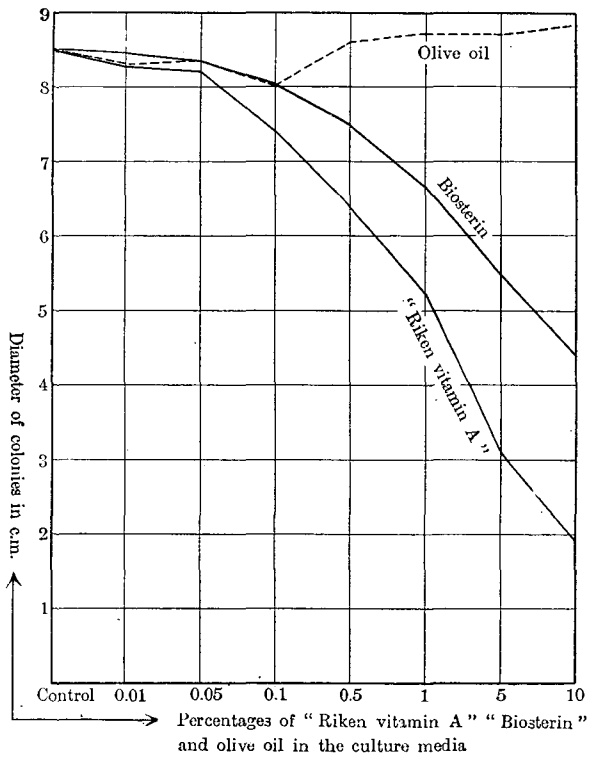
Fig. VI. The growth of *Helminthosporium turcicum* on culture media containing "Biosterin" in various percentages. (Data obtained in the first experiment.)



In these cultural experiments "Riken vitamin A" was used as the source of vitamin A, and hereafter "Biosterin" was omitted, because it seemed to have no other effect than "Riken vitamin A" upon the growth of fungi. Parallel to the main culture series of vitamin A a series of cultures containing pure olive oil have been carried out for comparison. The preparation of culture media and the entire operation were according to the methods described already in the foregoing chapter. The experiments were continued for 8 days due to the somewhat slower development of the fungus under test than that of the former species. At the end of this period the best growth occupied almost the entire space of the culture-plate. All the experiments were repeated twice.

The data obtained in the daily measurements of the diameters

Fig. VII. Curves comparing the growth of *Helminthosporium turcicum* developed in 6 days on the culture media containing olive oil, "Riken vitamin A", and "Biosterin" in various percentages.



of the colonies and the growth-curves of the fungus in relation to either duration of culture or concentrations of vitamin A and olive oil are shown in tables V and VI and figures VIII–X.

On the culture media containing olive oil the growth of the fungus has been observed to be favored greatly by the increase of its content. The rates of the increase in hypal development seemed to be greater at the medium percentages than at either low or high extremes in the present series of cultures ranging from 0.01% to 10%. Such an increase of hyphal growth owing to the addition of olive oil has been observed only in the case of *Ophiobolus Miyabeanus,* while in the cases of the other three species of fungi examined in the present studies olive oil contained in the culture medium seemed not to affect their growth. It is to be concluded that olive oil favors the growth of *Ophiobolus*

TABLE V.—*Average diameter in c.m. of colonies of Ophiobolus Miyabeanus developed on culture media containing olive oil in various percentages*

(a) First Experiment

Content of olive oil \ Duration of culture in days	1	2	3	4	5	6	7	8
Control	0.53	0.96	1.63	2.36	3.03	3.63	4.30	4.79
0.01 %	0.43	0.93	1.80	2.48	3.24	3.86	4.43	4.98
0.05 %	0.45	1.08	1.98	2.61	3.30	4.00	4.50	5.06
0.1 %	0.40	1.16	2.20	2.80	3.60	4.50	5.13	5.40
0.5 %	0.43	1.26	2.40	3.20	3.96	4.62	5.30	5.84
1 %	0.43	1.43	2.56	3.43	4.50	5.36	6.20	6.96
5 %	0.36	1.63	2.76	3.76	4.66	5.85	7.03	7.96
10 %	0.36	1.80	2.93	4.33	5.24	6.26	7.23	8.20

(b) Second Experiment

Content of olive oil \ Duration of culture in days	1	2	3	4	5	6	7
Control	0.63	1.30	1.96	2.80	3.36	3.86	4.66
0.01 %	0.70	1.40	2.10	3.03	3.63	4.30	4.96
0.05 %	0.63	1.36	2.06	2.86	3.53	4.10	4.90
0.1 %	0.56	1.43	2.16	2.96	3.70	4.36	5.03
0.5 %	0.56	1.70	2.46	3.80	4.40	5.30	6.46
1 %	0.73	1.76	2.70	4.13	4.86	5.93	7.16
5 %	0.53	1.80	2.86	4.30	5.33	6.30	7.37
10 %	0.53	1.90	3.06	4.43	5.56	6.53	7.73

Miyabeanus in the present culture medium under the present cultural conditions. The fungus colonies showed olivaceous black color in every culture.

The formation of conidia was observed in all cultures excepting the cultures containing 10% of olive oil, and it was more or less prominent in the majority of the cultures excepting those containing 5%

TABLE VI.—*Average diameter in c.m. of colonies of Ophiobolus Miyabeanus developed on culture media containing "Riken vitamin A" in various percentages*

(a) First Experiment

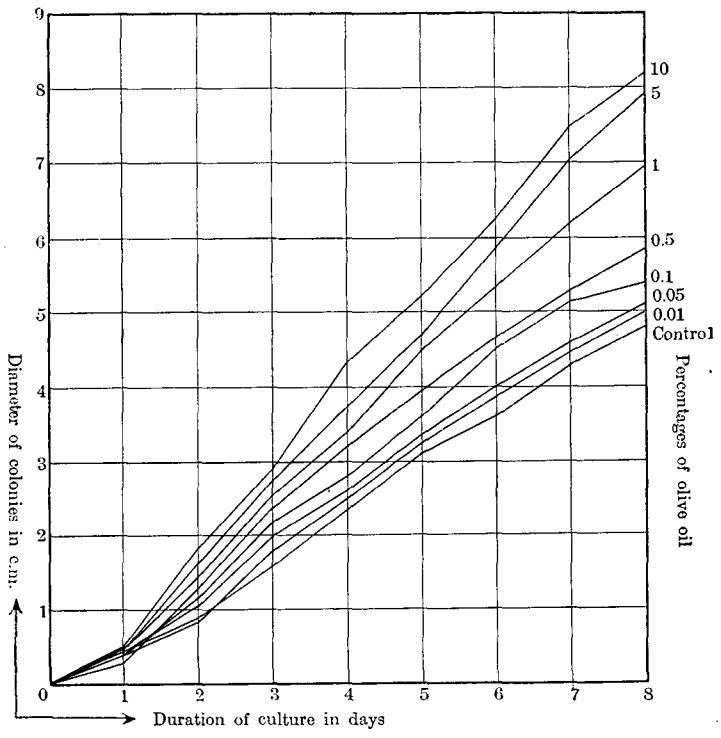
Content of Riken vitamin A \ Duration of culture in days	1	2	3	4	5	6	7	8
Control	0.60	1.33	2.00	2.70	3.13	4.00	4.53	5.10
0.01 %	0.53	1.30	1.86	2.60	3.20	3.96	4.56	5.18
0.05 %	0.56	1.43	2.13	2.96	3.63	4.43	5.13	5.83
0.1 %	0.60	1.50	2.40	3.36	4.03	4.93	5.73	6.24
0.5 %	0.53	1.66	2.56	3.53	4.36	5.33	6.10	6.73
1 %	0.42	1.60	2.46	3.46	4.10	5.03	5.80	6.36
5 %	0.20	1.40	1.96	2.66	3.30	4.14	4.73	5.26
10 %	0.10	0.93	1.00	1.10	1.16	1.20	1.26	1.50

(b) Second Experiment

Content of Riken vitamin A \ Duration of culture in days	1	2	3	4	5	6	7	8
Control	0.50	1.06	2.03	2.86	3.63	4.43	5.10	5.73
0.01 %	0.56	1.06	2.10	2.90	3.60	4.46	5.13	5.76
0.05 %	0.56	1.13	2.10	2.96	3.66	4.53	5.20	5.80
0.1 %	0.50	1.16	2.06	3.00	3.73	4.53	5.30	5.93
0.5 %	0.46	1.36	2.23	3.20	4.00	4.73	5.60	6.13
1 %	0.46	1.33	2.23	3.10	2.90	4.66	5.30	6.10
5 %	0.20	1.20	1.66	2.03	2.40	2.90	3.40	4.16
10 %	0.20	1.10	1.23	1.23	1.26	1.30	1.36	1.46

of olive oil, on which the conidia were produced a little. It is of consequence to note that the formation of conidia was retarded or completely checked in the cultures containing olive oil in higher percentages such as 5% or 10%, while the hyphal development was observed to be greatly favored in those cultures. In accordance with the general idea of the retardation of spore-formation of fungi being caused fre-

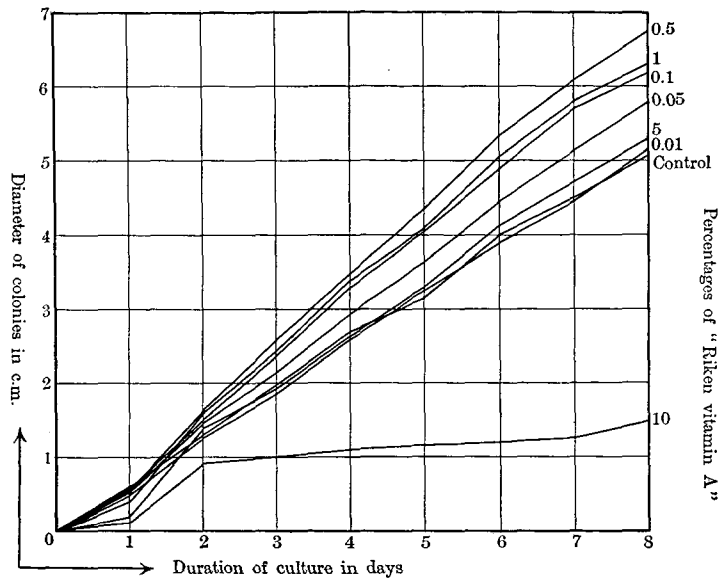
Fig. VIII. Growth of *Ophiobolus Miyabeanus* on culture media containing olive oil in various percentages. (Data obtained in the first experiment.)



quently by abundant nutrition, it is understood that the present cases of vigorous hyphal growth accompanying the retardation of conidia-production was caused by the especially high nutritive value of olive oil to *Ophiobolus Miyabeanus*. Such a remarkable requirement of fat or oil seems to be special to the present fungus and it is a rare case in the nutrition of fungi.

In the series of cultures containing vitamin A in various proportions, the diameter of colonies was greater in the cases of its lower content than in the control cultures, and the maximum was reached at 0.5% of "Riken vitamin A." Beyond this point, 0.5%, the diameter of the colonies decreased and marked retardations have been observed in the cultures containing higher proportions such as 5% or 10%. The average diameter of colonies developed in the cultures containing 10% of "Riken vitamin A" was less than one fourth of that of the maximal

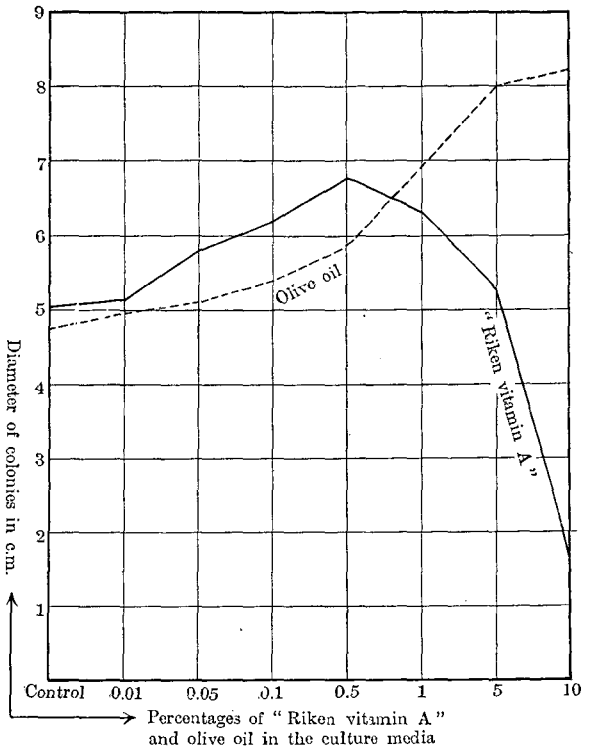
Fig. IX. Growth of *Ophiobolus Miyabeanus* on culture media containing "Riken vitamin A" in various percentages. (Data obtained in the first experiment.)



growth obtained in its 0.5% cultures or one third of that of the control cultures. Area of circle being in direct proportion to the square of radius, the differences occurred really among those normal or superior and retarded hyphal growths should be very great. As "Riken vitamin A" contains about 1.2% of vitamin A in olive oil, the actual content of vitamin A in every culture medium is approximately calculable. The maximum growth was obtained in the culture containing 0.5% of "Riken vitamin A," and marked retardation of growth was caused by 10% of "Riken vitamin A." Inspecting the growth obtained in the second experiment (Table V, b.), it is obvious that the negligibly slight increases of fungus growth have been caused by the increase of vitamin A content until 1% of "Riken vitamin A" was reached, and beyond this point a marked retardation of growth took place as result of its increase.

Olive oil having a special nutritive value for the present fungus, it seems to be somewhat difficult to decide simply whether the more or less superior growth of the fungus obtained in the cultures containing vitamin A in lower proportions was caused by vitamin A itself

Fig. X. Diameter of colonies of *Ophiobolus Miyabeanus* developed on culture media containing olive oil or "Riken vitamin A" in various percentages in 8 days old cultures.



directly or by olive oil nutritionally. The marked retardations observed in the cultures containing vitamin A in higher percentages, however, should be noticed as the effect of nothing other than vitamin A itself. In those higher percentages, as stated above, olive oil still greatly favored the hyphal development thus differing widely from the cases of vitamin A. The authors are inclined to conclude that vitamin A has no marked influence upon the development of the fungus either in acceleration or in retardation in lower percentages than 1% of "Riken vitamin A," but it retards the growth very strongly in percentages higher than this point. In the preceding experiments with *Helminthosporium turcicum* the evident retardation of growth took place at 0.1%, but in the present cases of *Ophiobolus Miyabeanus* marked retardation was observed first beyond 1% of "Riken vitamin A." The occurrence of such difference between these two kinds of fungi seems

to be due to the specific inequality of their nutritive response to olive oil, and in the case of *Ophiobolus Miyabeanus* it is possively inferred that the growth-retarding effect owing to vitamin A has been compensated to a certain extent by its specific nutritive behavior of olive oil, and beyond a certain critical point the supernutrition owing to olive oil having been surpassed by the unfavorable influence of vitamin A the retardation of growth was resulted. Accordingly one comes to the conclusion that the influence of vitamin A upon *Ophiobolus Miyabeanus* (=*Helminthosporium Oryzae*) is essentially unfavorable to its hpyhal development being theoretically analogous to the case of *Helminthosporium turcicum*.

Experiments on the Influences of Vitamin A upon the Growth of *Gibberella Fujikuroi*

The experiments having been carried out of two different species within the genus *Helminthosporium*, it would be interesting to extend the investigation to other kinds of fungi. The fungus under tests in the present experiments ought to be different systematically from the fungi mentioned above, but more or less similar to them in nutritional behavior. Under these considerations we preferred *Gibberella Fujikuroi* (=*Lisea Fujikuroi*) which belongs to the genus *Fusarium* in its conidial stage. This fungus is parasitic on rice plant, and it has a particular pathogenicity to the host plant. According to ITO and SHIMADA (1931) the fungus produces a certain special substance which causes hypertrophic elongation and chromatic hypogenesis of the cells of the host under either natural or artificial conditions, and the casual symptom appears as the elongation of the seedling accompanied with chlorosis to some extent.

The preparation of culture media and the conditions of culture were similar to the foregoing experiments. The series of cultures of "Riken vitamin A" and of pure olive oil have been carried out in parallel and the whole culture operations were repeated twice. The observation of growth and measurement of the diameter of colonies have been done every day until 8 days passed from the inoculation at which time the best growth occupied the whole space of the culture-plate. The daily increase of the diameter of colonies and their final differences due to the proportions of olive oil or "Riken vitamin A" in the culture media are shown in the following tables and figures:

TABLE VII.—*Average diameter in c.m. of colonies of Gibberella Fujikuroi developed on culture media containing olive oil in various percentages*

(a) First Experiment

Content of olive oil \ Duration of culture in days	1	2	3	4	5	6	7	8
Control	0.36	1.00	1.66	2.50	3.20	4.16	5.03	6.16
0.01 %	0.40	1.03	1.66	2.50	3.43	4.26	5.13	6.20
0.05 %	0.36	1.06	1.76	2.56	3.43	4.26	5.26	6.40
0.1 %	0.40	1.03	1.73	2.50	3.30	4.23	5.16	6.36
0.5 %	0.43	1.06	1.76	2.70	3.50	4.26	5.30	6.40
1 %	0.40	1.06	1.80	2.66	3.43	4.30	5.20	6.26
5 %	0.40	1.00	1.86	2.73	3.56	4.50	5.43	6.50
10 %	0.40	0.96	1.80	2.76	3.60	4.56	5.46	6.56

(b) Second Experiment

Content of olive oil \ Duration of culture in days	1	2	3	4	5	6	7	8
Control	0.43	1.40	2.33	3.16	3.90	4.70	5.50	6.20
0.01 %	0.46	1.46	2.43	3.26	4.03	4.96	5.96	6.76
0.05 %	0.56	1.43	2.46	3.40	4.13	5.03	6.03	6.83
0.1 %	0.60	1.46	2.50	3.43	4.23	5.10	6.16	7.06
0.5 %	0.56	1.50	2.63	3.56	4.36	5.23	6.33	7.33
1 %	0.56	1.46	2.53	3.43	4.30	5.20	6.16	7.23
5 %	0.50	1.26	2.16	3.20	3.90	4.66	5.56	6.43
10 %	0.56	1.26	2.13	3.13	3.86	4.50	5.36	6.26

The hyphal growths of the fungus on the culture media containing olive oil in varying percentages have been observed to be almost equal to the control cultures and also to each other. In other words the development of the fungus was apparently indifferent to the addition of olive oil in the culture media. Mycelium developed well in every culture and most of the colonies occupied the whole space of the

TABLE VIII.—*Average diameter in c.m. of colonies of Gibberella Fujikuroi developed on culture media containing "Riken vitamin A" in various percentages*

(a) First Experiment

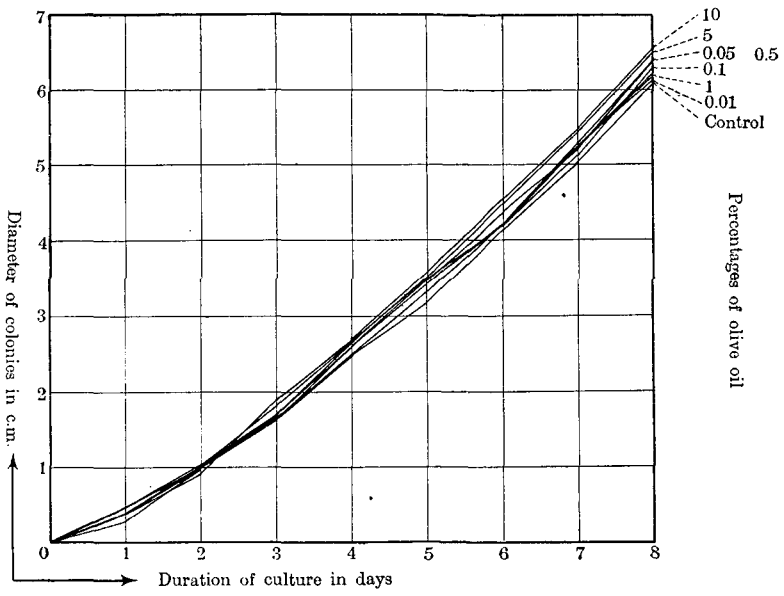
Content of Riken vitamin A \ Duration of culture in days	1	2	3	4	5	6	7	8
Control	0.63	1.36	2.30	3.23	4.03	4.80	5.66	6.40
0.01%	0.73	1.60	2.66	3.56	4.30	5.13	6.06	6.86
0.05%	0.63	1.63	2.80	3.73	4.66	5.56	6.63	7.66
0.1 %	0.70	1.56	2.70	3.76	4.76	5.73	6.76	7.73
0.5 %	0.60	1.40	2.56	3.46	4.16	5.00	5.90	6.76
1 %	0.63	1.20	2.26	3.20	3.90	4.73	5.56	6.36
5 %	0.53	1.16	2.00	2.66	3.30	3.93	4.66	5.23
10 %	0.43	1.03	1.73	2.43	3.00	3.60	4.16	4.83

(b) Second Experiment

Content of Riken vitamin A \ Duration of culture in days	1	2	3	4	5	6	7	8
Control	0.40	1.00	1.73	2.50	3.20	4.03	4.83	5.83
0.01%	0.40	1.16	1.93	2.80	3.76	4.73	5.73	6.73
0.05%	0.43	1.06	2.03	2.83	3.83	4.76	5.80	6.83
0.1 %	0.40	1.03	1.90	2.76	3.60	4.53	5.46	6.46
0.5 %	0.43	1.03	1.90	2.60	3.36	4.23	5.00	5.86
1 %	0.50	0.90	1.70	2.40	3.13	3.80	4.73	5.60
5 %	0.33	0.76	1.60	2.16	2.76	3.40	4.16	4.73
10 %	0.43	0.80	1.53	2.13	2.60	3.30	4.00	4.60

culture-plate within eight days from the inoculation. The creeping mycelium showed generally barium yellow color in the cultures containing olive oil in lower percentages. In the cultures containing 0.5% and 1% of olive oil, however, it showed orchraceous orange color, and in the latter case several concentric rings of lilac color were observed. In the cultures containing 5% and 10% of olive oil concentric colora-

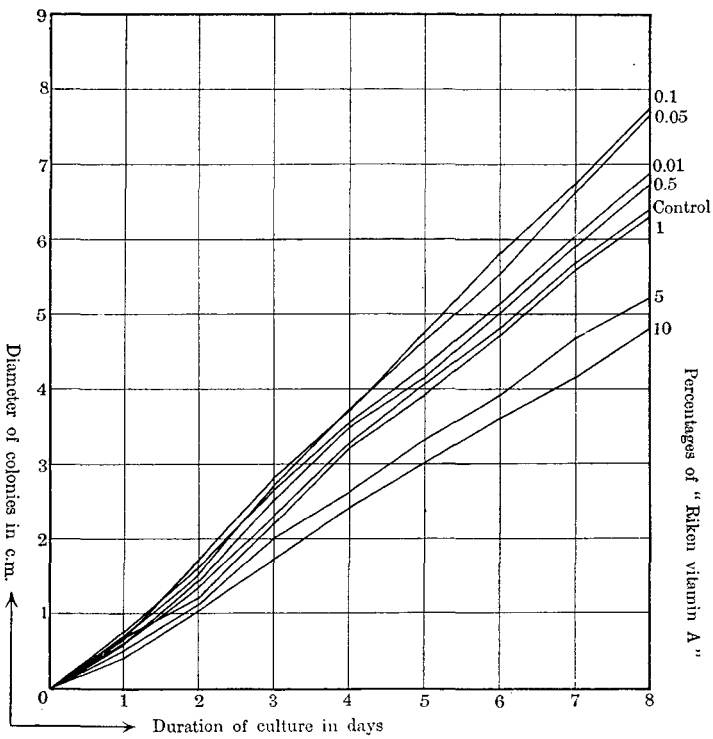
Fig. XI. Growth of *Gibberella Fujukuroi* on culture media containing olive oil in various percentages. (Data obtained in the first experiment.)



tions of chinese violet deeper toward the marginal area of the colonies were observed. The aerial mycelium developd well in every culture showing snowy white in color. The production of conidia has been observed slightly in every case.

In the cases of the cultures containing vitamin A the hyphal growth of the fungus seemed to be favored by its lower content but retarded by its higher content in the culture medium. The fungus showed better developments in the cultures containing 0.01%, 0.05% and 0.1% of "Riken vitamin A" than in the control cultures, and the maximum growths were attained at 0.1% in the first experiment and at 0.05% in the second. In the cultures containing 0.5% and 1% of "Riken vitamin A," however, the growth of the fungus was almost similar to the control cultures. In the cultures containing 5% and 10% of "Riken vitamin A," however, the growth of the fungus was clearly retarded. From the results of the experiments it is concluded that the hyphal growth of *Gibberella Fujikuroi* is favored by lower content of vitamin A in the culture medium but it is retarded by its higher content. In other words the effect of vitamin A upon the growth of

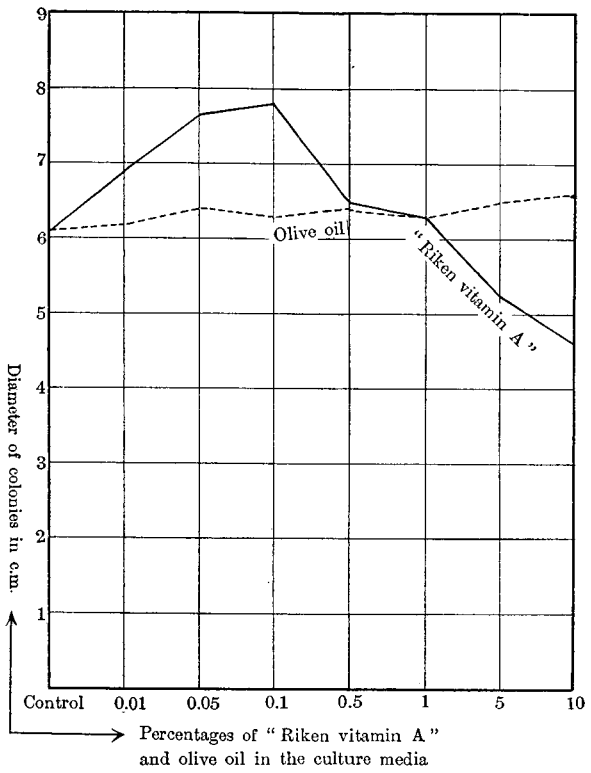
Fig. XII. Growth of *Gibberella Fujikuroi* on culture media containing "Riken vitamin A" in various percentages. (Data obtained in the second experiment.)



the fungus is variable from acceleration to retardation according to the increase of its volumes contained in the culture medium. The transition point of the effect of vitamin A seems to lie between 0.5% to 1% as "Riken vitamin A" where the growth of the fungus was observed to be almost similar to the control cultures which contain no vitamin A.

The creeping mycelia developed in the culture media containing 0.01%, 0.05% and 0.1% of "Riken vitamin A" and in the control cultures showed a partial mixing of primrose yellow and honey yellow in color but those developed on the cultures containing more than 0.5% of "Riken vitamin A" showed ochraceous orange color. The difference observed between the colorations of creeping mycelium developed in the cultures containing vitamin A in lower percentages and those of higher percentages suggest a correlation between the promoting

Fig. XIII. Curves comparing the growth of *Gibberella Fujikuroi* developed in 8 days on culture media containing olive oil and "Riken vitamin A" in various percentages.



or retarding effects of vitamin A and the colorations. In other words it can be said that the addition of "Riken vitamin A" over than 0.5% in the culture medium presents a retarding effect on the hyphal growth of the fungus accompanied by a change of the coloration of creeping mycelium from primrose yellow or honey yellow to ochraceous orange. It may be interpreted that the ochraceous orange coloration of the creeping mycelium is an indication of the unfavorable effect of vitamin A upon the fungus in addition to the retardation of the development of the mycelial colonies. The aerial mycelium, however, developed well in every culture and showed similarly snow white color. The production of conidia was observed slightly in all the cultures and no marked influence at all was recognized owing to the difference of the content of vitamin A in the culture medium.

Experiments on the Influence of Vitamin A upon the Growth of *Glomerella Lindemuthiana*

The fungus under test in the present experiments, *Glomerella Lindemuthiana,* which causes the anthracnose of beans has been known as *Colletotrichum Lindemuthianum* in its conidial stage. It is quite different from the three kinds of fungi examined in the foregoing experiments in its nutritional behaviors.

The experiments have been carried out with "Riken vitamin A" and pure olive oil in exactly similar ways to the previous cultural experiments with the one exception of eliminating 10% "Riken vitamin A" culture in the present series. The observations and measurements of the development of the hyphal colonies have been made every other day because the growth of the fungus was too slow to measure daily. The best growth occupied almost the whole surface of the culture-plate only after twenty-four days from the inoculation.

The diameters of the fungus colonies measured every other day and their final differences observed on the 24th day of the experiment connecting with the proportions of olive oil or "Riken vitamin A" in the culture media are shown in the following tables and figures:

TABLE IX.—*Average diameter in c.m. of colonies of Glomerella Lindemuthiana developed on culture media containing olive oil in various percentages*

Content of olive oil \ Duration of culture in days	2	4	6	8	10	12	14	16	18	20	22	24
Control	0.4	1.0	1.6	2.1	2.5	3.1	3.5	3.8	4.3	4.8	5.2	5.7
0.01 %	0.5	1.2	1.9	2.6	3.1	3.7	4.2	4.5	5.3	5.7	6.1	6.4
0.05 %	0.6	1.3	1.9	2.5	2.9	3.5	4.0	4.5	5.2	5.8	6.5	7.0
0.1 %	0.5	1.3	2.0	2.5	2.9	3.3	4.0	4.6	5.1	5.5	6.2	6.8
0.5 %	0.5	1.3	1.9	2.6	4.0	3.6	4.1	4.6	5.2	5.5	6.2	6.7
1 %	0.5	1.3	2.0	2.7	3.2	3.7	4.3	4.9	5.4	5.8	6.3	6.9
5 %	0.5	1.2	1.8	2.4	2.8	3.3	3.9	4.4	4.9	5.3	5.8	6.4
10 %	0.5	1.4	2.1	2.7	3.0	3.5	4.3	4.7	5.2	5.5	6.0	6.5

TABLE X.—*Average diameter in c.m. of colonies of Glomerella Lindemuthiana developed on culture media containing "Riken vitamin A" in various percentages*

Content of Riken vitamin A \ Duration of culture in days	2	4	6	8	10	12	14	16	18	20	22	24
Control	0.5	1.1	1.7	2.2	2.5	3.1	3.6	3.9	4.5	4.9	5.5	5.9
0.01 %	0.6	1.0	1.5	1.9	2.3	2.8	3.3	3.5	4.0	4.3	5.0	5.6
0.05 %	0.5	1.0	1.4	1.8	2.2	2.6	3.0	3.3	3.8	4.2	4.9	5.4
0.1 %	0.5	1.0	1.4	1.7	2.1	2.4	2.8	3.2	3.6	4 0	4.5	5.2
0.5 %	0.5	0.9	1.2	1.6	1.9	2.3	2.6	3.0	3.4	3.8	4.3	4.8
1 %	0.3	0.8	1.0	1.5	1.7	2.2	2.5	2.6	2.9	3.5	4.0	4.5
5 %	0.4	0.4	0.5	0.6	0.6	0.6	0.6	0.6	0.6	0.7	0.7	0.8

Fig. XIV. Growth of *Glomerella Lindemuthiana* on culture media containing olive oil in various percentages.

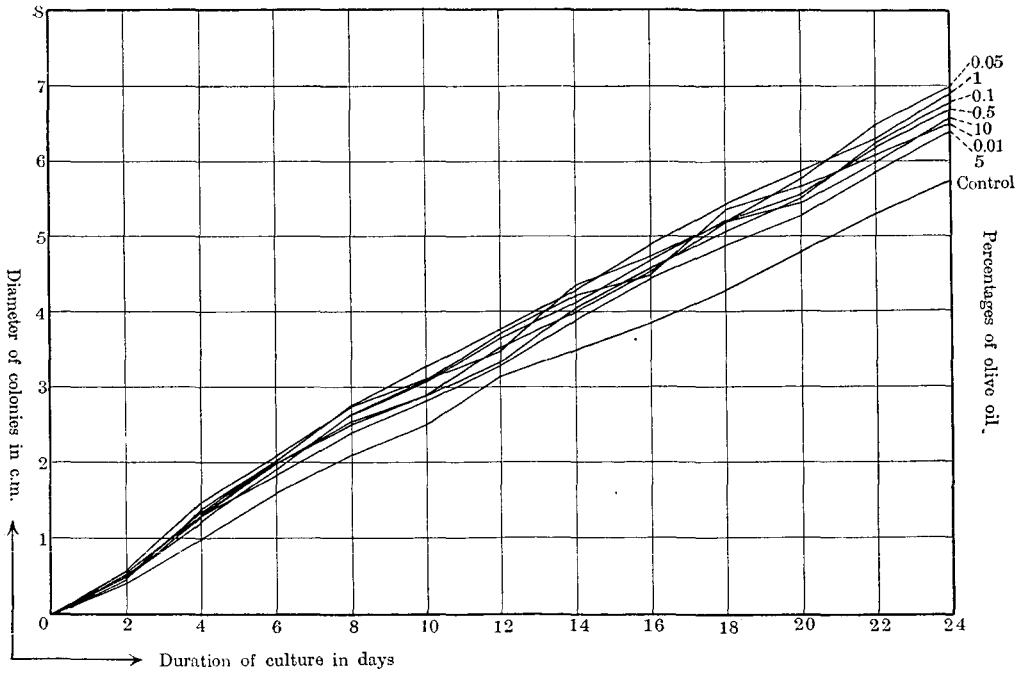
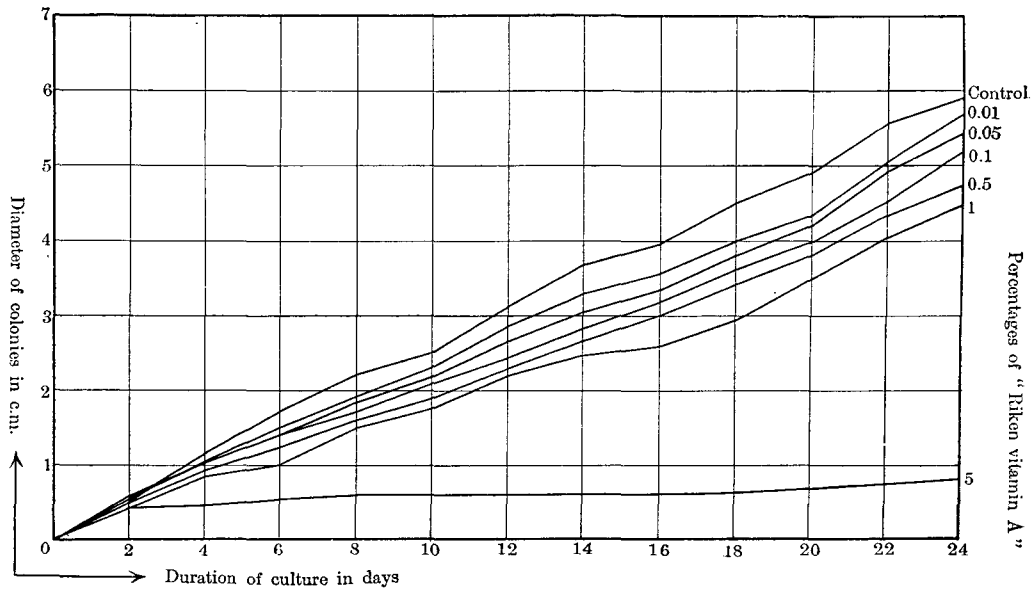


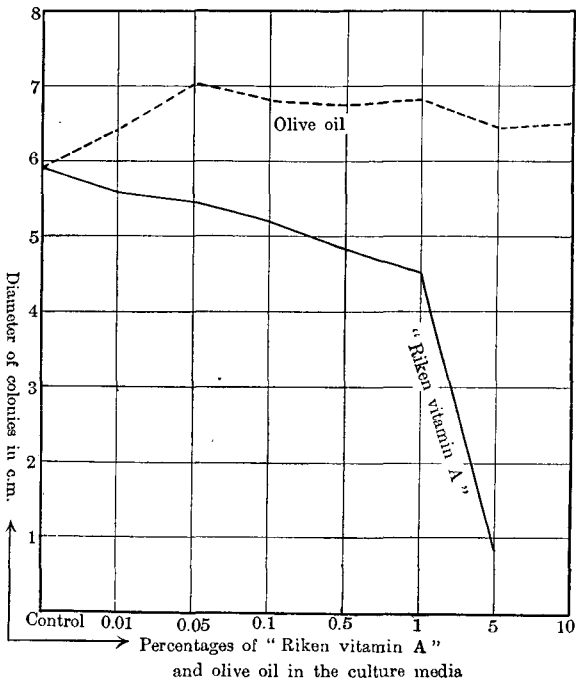
Fig. XV. Growth of *Glomerella Lindemuthiana* on culture media containing "Riken vitamin A" in various percentages.



In the series of olive oil cultures the hyphal development of the fungus on every culture containing pure olive oil in the proportions ranging from 0.01% to 10% was more or less better than the control cultures without olive oil. The best growth was attained in the 0.05% culture, but there has been observed no marked difference among the cultures differing in the content of olive oil. In the control cultures the fungus developed into compact mycelial colonies colored olivaceous black with almost no aerial growth. The development of aerial mycelia seemed to increase proportionately to the content of olive oil in the culture medium, and it covered almost all the surface of the colony in the cultures containing 5% and 10% of olive oil. The production of conidia was abundant in all cultures.

In the cultural series of "Riken vitamin A" the best growth was attained in the control cultures and the hyphal development of the fungus was retarded gradually by the increase of the "Riken vitamin A" in the culture media until it reached 1%. Beyond this point, however, the retardation of growth became quite remarkable and in the 5% cultures the diameter of the colonies reached hardly to 1 c.m.

Fig. XVI. Curves comparing the growth of *Glomerella Lindemuthiana* developed in 24 days on culture media containing olive oil and "Riken vitamin A" in various percentages.



even after 24 days' incubation. It would be obviously concluded from the cultural results that the existence of vitamin A in the culture medium is unfavorable to the hyphal development of the fungus under question even in a so small amount as 0.01% of "Riken vitamin A."

In every culture the white aerial mycelia developed well covering almost all the surface of the colonies. Comparing them to the control cultures on which no aerial mycelia developed, it may be said that "Riken vitamin A" favors the development of aerial growth of the fungus. Even in the cases of the very poor hyphal development in the cultures containing 5% of "Riken vitamin A" the white aerial growth was clearly observed. It can not be easily decided, however, whether the luxuriant development of the aerial mycelium was caused really by vitamin A itself or by olive oil, because the aerial mycelia developed well not only in the cultures containing "Riken vitamin A" but also in those containing olive oil only.

Discussion

The fat-soluble vitamin A has been known as an indispensable substance in the normal growth of animals and also to have a curing effect upon certain avitaminoses just as to the other vitamins. The effect of vitamins upon fungi should be an interesting study. The problem has been studied of the effect of the water-soluble vitamin B mostly upon the fungi concerned in agricultural technology but very little has been done on plant pathogenes. As far as the authors know the effects of the fat-soluble vitamin A on the development of parasitic fungi have not been reported heretofore. In the present studies the effects of vitamin A on four kinds of fungi, namely *Helminthosporium turcicum, Ophiobolus Miyabeanus, Gibberella Fujikuroi* and *Glomerella Lindemuthiana* have been examined.

In every examination of the four kinds of fungi retardation of growth was caused by an addition of vitamin A in the culture medium. In the cases of *Ophiobolus Miyabeanus* and *Gibberella Fujikuroi,* however, an apparent growth-promotion was observed in the cultures containing "Riken vitamin A" in lower proportions such as 0.5% in the former species and 0.05% to 0.1% in the latter.

It cannot be decided certainly whether the growth-promotion observed in the cases of *Ophiobolus Miyabeanus* has been caused really by vitamin A or by olive oil. As the source of vitamin A used in the present studies, "Riken vitamin A," is an olive oil solution of vitamin A extracted from cod-liver oil, the influence of olive oil itself should be considered. Therefore the cultural experiments on the culture media containing pure olive oil were carried out parallel to those with cultures of vitamin A. In general, no marked effect of olive oil has been observed in any cultures excepting those of *Ophiobolus Miyabeanus.* This fungus seemed to have an unusual nutritive speciality to olive oil, and a remarkable promotion of hyphal growth has been observed in the cultures containing pure olive oil in proportion to its increase of percentage in culture medium. Therefore the results obtained in the cultural studies of "Riken vitamin A" which contains about 1.2% of vitamin A in olive oil should be contemplated bearing in mind the effects of olive oil as well as those of vitamin A. Consequently the growth-curve obtained in the "Riken vitamin A" cultures (Fig. IX, X) represents the compensated results of the growth-promoting effects of olive oil and the reverse effects of vitamin A. In the cultures of

higher limit of vitamin A content the hyphal growth of the fungus was retarded greatly, and it is easily understood that the favorable nutritive effects of olive oil were overwhelmed by the growth-retarding effects of vitamin A. In the cases of the cultures containing comparatively lower percentages of "Riken vitamin A," however, the resulting growth-promotion was caused either by the effects of olive oil or vitamin A or both in combination. The authors are inclined to believe that "Riken vitamin A" has some favorable influences upon the hyphal development of the fungus in its lower contents in the culture medium, but having a demarcation point somewhere between 0.5% to 1% beyond which it acts unfavorably.

In the case of *Gibberella Fujikuroi,* olive oil having no marked influence upon the development of the fungus, it is clearly understood that the growth-promotion resulting in the cultures containing "Riken vitamin A" in lower percentages is caused by the effects of vitamin A itself. In this case the demarcation point lies at nearly 0.1% beyond which an unfavorable effect appeared.

In the cases of *Helminthosporium turcicum* and *Glomerella Lindemuthiana* the effects of vitamin A were uttedly unfavorable within the range of percentage examined in the present cultural studies. A retardation in the development of the fungi was observed clearly with a slight existence of vitamin A in the culture medium.

Thinking over the results obtained in the present series of cultural studies in general, it is obvious that vitamin A causes retardation in the growth of the fungi by the addition of certain amounts of it to the culture medium. The amount of vitamin A at which the retarding effect on hyphal development begins is different with different fungi, *i. e.* even so small amount of "Riken vitamin A" as 0.01% for *Helminthosporium turcicum* and *Glomerella Indemuthiana,* 0.1% for *Gibberella Fujikuroi,* and 1% for *Ophiobolus Miyabeanus.*

Retardation of growth in animals caused by vitamin D has been reported by COLLAZO and co-workers (1929). They fed rats with vitamin D obtained by the treatment of ergosterin with ultraviolet ray, and observed a severe case of hypervitaminose of the animals. Vitamin D has been distinguished recently from so-called vitamin A by MCCOLLUM and co-workers (1922) as a special principle being concerned with the metabolism of calcium in constituting bones. The sources of vitamin A used in the present studies, "Riken vitamin A" and "Biosterin," have been considered to contain so-called vitamin D

according to the process of preparation and feeding tests of animals. Therefore it is hard from our results to determine whether the retardation of the growth of the fungi under question was caused by vitamin A or by vitamin D.

The present authors would like to conclude that the fat-soluble vitamins or vitamin A in a broad sense cause or causes occasional promotion and ultimate retardation of the development of the fungi under question.

Although a case of hypervitaminose caused by vitamin D was reported by COLLAZO and co-workers, the vitamins having been considered in general as the substances promoting the growth of animals and even some kinds of microorganisms, the present conclusions seem to be interesting and something unusual, differing from the general conceptions of the effects of vitamins hitherto current.

Summary

Although a great number of studies on the nutritive effects of vitamins have been published, only a few have reported the effect of vitamin B and none have considered that of vitamin A upon parasitic fungi. The present studies were intended to learn the effects of so-called vitamin A or the fat-soluble vitamins on the development of four kinds of plant-pathogenic fungi, namely *Helminthosporium turcicum, Ophibolus Miyabeanus, Gibberella Fujikuroi* and *Glomerella Lindemuthiana*. As the sources of vitamin A "Riken vitamin A" and "Biosterin" which are prepared and sold by the Physical and Chemical Research Institute of the Japanese Government were used.

Vitamin A is known to be fairly stable to high temperature. We have tested the thermo-stability of "Riken vitamin A" by way of precaution and proved that the sterilization of culture media containing "Riken vitamin A" in various percentages at 100°C. in KOCH's steam sterilizer gave no worse effect than the intermittent sterilization at 68°–70°C.

"Riken vitamin A" and "Biosterin" being olive oil solutions of so-called vitamin A extracted from cod-liver oil in the concentrations about 1.2% and 1.5–2.0% respectively, the cultures of olive oil have been carried out parallel to those of "Riken vitamin A" and "Biosterin."

The growth of *Helminthosporium turcicum* seemed to be quite indifferent to an addition of olive oil in the culture medium, but addition both of "Riken vitamin A" and of "Biosterin" had a remarkable retarding effect on the hyphal development.

In the cases of *Ophiobolus Miybeanus* (*Helminthosporium Oryzae*), however, olive oil promoted the growth of the fungus greatly. It is interesting to note that this fungus seems to have a special nutritive behavior to olive oil. "Riken vitamin A" seemed to promote the growth of the fungus in its lower percentages, while the development of the fungus was retarded markedly in the higher percentages. It can not be easily said, however, whether the growth-promotion presented in the cultures containing "Riken vitamin A" in lower percentages was caused really by vitamin A or by olive oil.

Gibberella Fujikuroi seemed to grow indifferently to olive oil contained in the culture medium, but "Riken vitamin A" promoted the hyphal growth in its lower percentages and retarded it in higher ones. In these cases the growth-promotion was clearly caused by vitamin A itself as well as the retardation on the growth in the cultures containing it in higher percentages. The effects of vitamin A upon the growth of the fungus changed from promotion to retardation accompanied by an alteration of the coloration of the creeping mycelium.

Glomerella Lindemuthiana (*Colletotrichum Lindemuthianum*) showed more or less better growth in the cultures containing olive oil especially in its lower percentages, and the production of aerial mycelia seemed to be promoted proportionately to the increase of of olive oil content. The "Riken vitamin A," however retarded the hyphal growth definitely even in the lowest percentage in the present series and nearly inhibited growth in the highest one.

It is concluded from the results obtained in the present cultural studies of four kinds of parasitic fungi concerning "Riken vitamin A" and "Biosterin" that the so-called vitamin A causes occasional growth-promotion in its lower contents in the culture medium but as a whole the ultimate effect of this substance upon the development of these fungi was nothing but a growth-retardation.

"Riken vitamin A" and "Biosterin" are generally considered as the source of so-called vitamin A, but it is highly probable according to the methods of their preparation that they contain a certain amount of vitamin D other than vitamin A or biosterin. Of vitamin D a case of hypervitaminose was reported by COLLAZO and

co-workers (1929), but vitamin A has been considered as a growth-promoting principle for animals and yeast-fungi. In the present studies, however, we observed the growth-retarding effect of the so-called vitamin A in broad sense upon the hyphal development of the fungi under examination, but it should be a problem of future investigation to decide whether the retardation of the growth of these fungi is caused by vitamin A in strict sense or by vitamin D.

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EXPLANATION OF THE PLATES

Fig. 1. Effects of "Riken vitamin A" and olive oil on the growth of *Helminthosporium turcicum*.
K. Control.
A. "Riken vitamin A", 10%.
B. Ditto, 1%.
C. Ditto, 0.1%.
A'. Olive oil, 10%.
B'. Ditto, 1%.
C'. Ditto, 0.1%.

Fig. 2. Effects of "Riken vitamin A" and olive oil on the growth of *Ophiobolus Miyabeanus*.
K. Control.
A. "Riken vitamin A", 10%.
B. Ditto, 1%.
C. Ditto, 0.1%.
A'. Olive oil, 10%.
B'. Ditto, 1%.
C'. Ditto, 0.1%.

Fig. 3. Effects of "Riken vitamin A" and olive oil on the growth of *Gibberella Fujikuroi*.
K. Control.
A. "Riken vitamin A", 10%.
B. Ditto, 1%.
C. Ditto, 0.1%.
A'. Olive oil, 10%.
B'. Ditto, 1%.
C'. Ditto, 0.1%.

Fig. 4. Effects of "Riken vitamin A" and olive oil on the growth of *Glomerella Lindemuthiana*.
K. Control.
A. "Riken vitamin A", 5%.
B. Ditto, 1%.
C. Ditto, 0.1%.
A'. Olive oil, 10%.
B'. Ditto, 1%.
C'. Ditto, 0.1%.

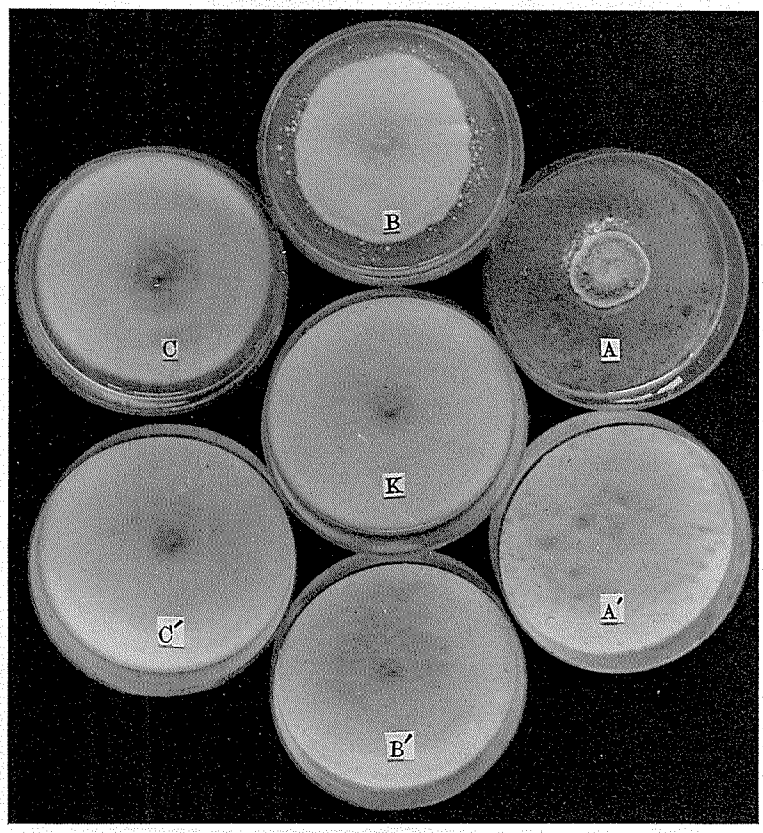

Fig. 1.

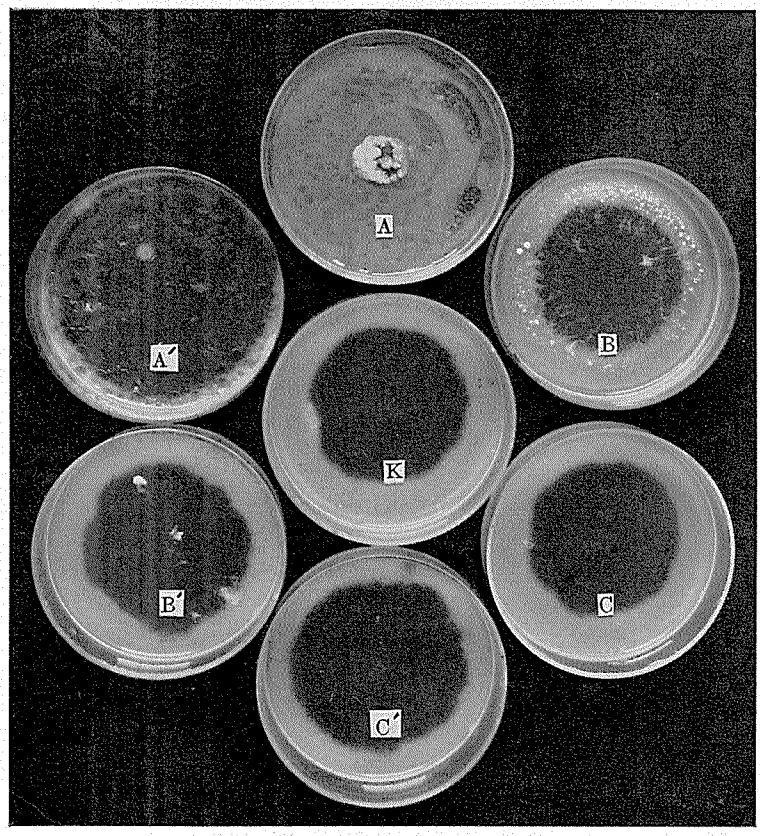

Fig. 2.

Fig 3.

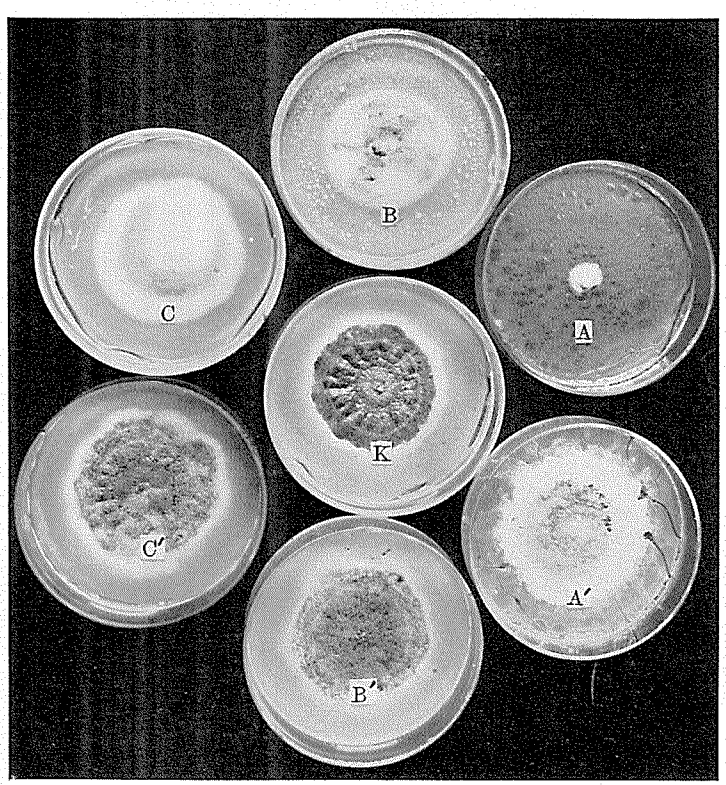

Fig 4.