The couple of 4u/ml and 1u/ml were considered as the standard and 2u/ml and 0.5u/ml as the test and this potency was calculated from the diameters of the inhibition zones and written in the eighth column of Table 3. From these results the mean error of the value obtained by using four plates was calculated. The error after three hours incubation is significantly larger than that after four or five hours. The standard deviation is 11% after three hours, 5.2% after four hours, and 6% after five hours.

D. Discussion.

The error in this rapid method is small enough for the potency test during the tank fermentation. But, as the diameters are not so large as in the case of the usual cup method, it is better to use the standard solution in the concentrations of 4u/ml and 1u/ml.

The inhibition zones after three hours are smaller than that after four or five hours. That is, the growing bacteria must lose the dehydrogenating activity by the action of penicillin. But it is usually confirmed that penicillin has no influence upon the dehydrogenation of bacteria suspended in glucose phosphate buffer solution. Yanagita (5) has observed that the growing bacteria are lysed by penicillin. Then it seems that the growing bacteria are lysed by penicillin and loses the dehydrogenating power.

SUMMARY

- 1) The standard deviation observed by using five plates was about 2% in our laboratory.
- 2) Miyamura's rapid method can show a fairly accurate potency in four hours incubation.

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A CRYSTALLINE ANTIBACTERIAL SUBSTANCE FROM PENICILLIUM LEUCOPUS AND FOUR OTHER STRAINS OF PENICILLIA, AND ASPERGILLUS CLAVATUS, AND ITS PROBABLE IDENTITY WITH PATULIN

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As we were searching strains for penicillin, two strains of Penicillia, antagonistic

against both gram negative and positive bacteria, were isolated from the air and their antibacterial substance was purified up to the colourless plate crystals. Afterwards we could also extract the same substance from three other strains of *Penicillia* isolated by other workers in Japan and sent to our laboratory, and also from *Aspergillus clavatus*.

In publication by Bergel and others (1) the chemical, physical, biological properties of Patulin, Clavatin and Claviformin and their identity were described. As the properties of the antibacterial substance obtained by us are similar to Patulin and it is extracted, not only from five strains of *Penicillia* but also from *Aspergillus clavatus*, it is highly probable that the substance obtained by us is identical with Patulin. After the publication of our work in Japanese (January, 1946), Kuroya informed us that he had also extracted the same substance from a strain of *Penicillium*. This substance has been obtained from many strains isolated from various places, so it must be widely distributed in nature.

I. About strains.

1) C1 and C2 strains (*Penicillia*) were isolated from the air in our laboratory and the taxonomic studies on C1 Strain (in cooperation with Dr. Dang-Van-Ngu, Assist. Prof. at Hanoi University and now studying in our laboratory) have been finished. The details are as follows.

Colonies on Czapeck-Dox agar blue-green, with broad white margin at the growing period. Gregarious coremia, 1-2mm high, 1mm in diameter, with white stalks and round heads, at first white then blue-green, aggregated in concentric zones quite sharply separated by furrows 2-3mm broad, sterile in some cultures (sulcate or suzotate condition), but with scattered simple conidiophores in others. Moldy odor strong. Drops uncoloured, abundant. Reverse yellow passing to brown in old cultures.

Conidiophores $1.5-2\mu$ in diameter, with smooth wall. *Penicillus* 50 to 100μ asymmetrically biverticillata, with 2-4 metulae bearing each 2-4 sterigmata, or monoverticillata with 1-4 sterigmata. Metulae $15-16\mu$ by 2μ . Sterigmata $10-18\mu$ by 2μ with apex acuminate $1.5-2\mu$ long. Conidia elliptical, at first $1.5-2\mu$ by 3μ becoming, when ripe, globose 3.5μ in diameter, smooth.

These characters of C1 strain correspond to those of *P. leucopus*, Biouge, 1923, described in Thom's book (1930, p. 408). The zonate feature when developed under favorable conditions, and the strong moldy odor justify its separation from *P. expansum* Thom.

2) A-8 strain (*Penicillium* sp.) was isolated from the rice cake by Aizawa at the Department of Hygiene of Tokyo University.

3) LHS-b5 strain (*Penicill. sp.*) was isolated from the air in Nagoya by Kubo, at the Department of Plant Physiology, Nagoya University.

4) A-7 strain was isolated from the air in Kochi-Prefecture by Miyakoda.

All these strains build the gregarious coremia and their culture fluid has the special odor resembling that of an apple.

A strain of *Aspergillus clavatus* was sent to our laboratory by Okunuki, Member of Iwata Institute for Plant Physiology in Tokyo.

II. Necessary conditions for production of the antibiotic substance.

In order to get the antibiotic substance it is necessary to cultivate the strains in sugar rich media, for example in Czapeck-Dox medium (glucose 4%) or the medium from beef extract 5g, NaCl 5g, glucose 50g and H₂O 1000ml. After about seven days at 23°–25°C the culture fluid of C1 (*P. leucopus*), C2, A-8, LHS-b5 and A-7 (all *Penicillium* strains) inhibits the growth of *B. coli* at dilution of 1:800–1600 and the fluid of *Aspergillus clavatus* at 1:80.

III. Purification.

This antibiotic substance is thermostable at pH 6.0, so the culture fluid is at first concentrated to its twentieth volume by means of vacuum distillation at about 70°C and from this concentrated solution the active constituent is extracted with ether. The ether solution is dehydrated with Na₂SO₄ and is distilled off until the glutinous mass containing the active element is left. From this glutinous mass the active constituent is extracted into absolute ether and after the ether is distilled off the crude active crystalline substance can be obtained. After recrystallization two or three times from ether or benzene the colourless platelet crystals of the antibiotic substance are obtained.

IV. The chemical and bacteriological characters.

All antibacterial substances from the several strains tested are the same, as they have the same melting point and the following characters. The melting point lies at 109.5°–110.5°C and the results of the elemental analysis (C 54.66%, H 3.88%, O 41.46%) and molecular weight determination (about 148) agree with the molecular formula C₇H₆O₄. It is easily soluble in acetone, ether, alcohol and water, but insoluble in petroleum ether and cold benzene and it makes phenylhydrazone (its melting point is 149°C). It has the pungent taste.

It is much more toxic than penicillin and its minimal lethal dose against mice (body weight about 10g) is about 0.4mg by subcutaneous injection.

Table 1.

	Claviformin Clavatin	Patulin	Leucopin (obtained by us)
Molecular formula	C ₇ H ₆ O ₄	C ₇ H ₆ O ₄	C ₇ H ₆ O ₄
Melting point	109.5–110.5°C	111°C	109.5–110.5°C
M.P. of phenylhydrazone	149–150°C	152–153°C	149°C
Antibacterial activity against <i>Staphylococcus aureus</i>	1 : 64,000–128,000	1 : 64,000–128,000	1 : 80,000–160,000

V. Discussion.

Glister (3) found the antibiotic substance against gram negative bacteria in the culture fluid of *Aspergillus*, and according to the review by Kiese (4) Wiesner also found the same substance and tried to purify it. Besides, afterwards, according to a short report

by Bergel and others (1), Raistrick obtained the crystalline antibiotic substance against gram positive and negative bacteria and called it Patulin from *P. patulum*, and Bergel and others obtained the same substance from *P. claviforme* (Claviformin) and *Asp. clavatus* (Clavatin). As may be seen in Table 1, these three substances have the same properties as the substance purified by us. So the substance obtained by us must be identical with Patulin from *P. patulum*, Claviformin from *P. claviforme* and Clavatin from *Asp. clavatus*.

SUMMARY

The antibiotic substance identical with Patulin was extracted from the culture liquid of *P. leucopus*, four other *Penicillia* and *Asp. clavatus*.

This experiment was performed at the Government Institute for Infectious Diseases and reported at its meeting, January, 1946.

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ISOLATION OF ACTINOMYCIN A FROM A STRAIN OF STREPTOMYCES

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Antagonism between *Actinomyces* and bacteria has been observed by many workers, and the literature is reviewed by Waksman (1). But it is rather recently that the antibiotic substances are extracted and purified up to the crystalline forms. According to the review by Kiese (2), Actinomycin A is the first crystalline antibiotic substance from *Actinomyces*.

Since we had begun to study the antibiotics from *Actinomyces* (January, 1946), the red crystalline antibiotic substance was first extracted. And owing to the contradictory descriptions in the reviews by Kiese (2), and by Hallauer (3), it could not be decided whether this substance was identical with Actinomycin A, until we had a chance to read the monograph concerning the antibiotics written by Waksman (4).

I. On the strain from which Actinomycin A was extracted.

This strain was isolated from the garden soil in our institute, and its characters are as follows:

Coarse filaments with branching hyphae. Conidia formed by budding and braking up