be observed that calcium pectinate gels containing 35 per cent sugar are formed only when the degree of esterification of the polygalacturonide chain is less than 50 per cent.

Data for samples A and A-1 show that by removal of nongalacturonide material the methoxyl value and the neutralization equivalent may be changed greatly, while the degree of esterification is reduced only

TABLE 1

DATA CHARACTERIZING TWO SERIES OF ACID—DE-ESTERIFIED PECTINS

Sample No.	Description	Ca-pectinate gel formation*	Per cent esterification	Per cent CH_3O	Neut. equiv.	Per cent galacturonide
A	Commercial apple pectin	None	68.0	6.57	990	57.8
A-1	Sample A purified†	None	66.7	8.06	769	71.1
A-2	Sample A acid de-esterified	None	50.6	6.61	476	78.3
A-3	Sample A acid de-esterified	Strong	38.5	5.23	370	80.1
B	Purified apple pectin†	None	74.2	9.36	952	76.0
B-1	Sample B acid de-esterified	Weak	45.2	6.78	391	85.7
B-2	Sample B acid de-esterified	Strong	24.2	3.70	269	89.1

* Calcium pectinate gels, containing 35 per cent sugar, prepared by method of Hills, White, and Baker (Hills, C. H., White, J. W., Jr., and Baker, G. L. *Proc. Inst. Food Tech.*, 1942, 47–58).

† Purified by dissolving in water, adding 5 per cent conc. HCl by volume, immediately precipitating with ethanol, and washing with ethanol until free of chlorides.

slightly. Clearly, the increase in the jelly grade of pectins upon mild acid de-esterification observed by Baker and Goodwin (*1*) may be caused in part by the removal of inactive constituents and the consequent increase in galacturonide content.

The degree of esterification and the galacturonide content may be calculated readily from the methoxyl value and titration data. Each anhydrogalacturonide residue in the chain may be considered to contain either a free carboxyl or a methyl-esterified carboxyl group.[2] The number of moles of free carboxyl groups (N) per gram of pectin may be determined from the alkalinity of the ash and the amount of alkali required to titrate an aqueous solution of pectin to pH 7.5 (*4*). The number of moles of methyl-esterified carboxyl groups may be determined by methyl ester analysis (*2*) and the formula

$$Z = \frac{\text{wt. per cent } CH_3O}{3100}$$

From these two quantities one may compute the per cent galacturonide, the per cent nongalacturonide, the neutralization equivalent, the per cent esterification of the galacturonide chain, and the average residue weight by the relations

$$\text{Per cent galacturonide} = (176\,N + 190\,Z) \times 100$$

$$\text{Per cent nongalacturonide} = 100 - \text{per cent galacturonide}$$

$$\text{Neutralization equivalent} = \frac{1}{N}$$

$$\text{Per cent esterification} = \frac{Z}{Z+N} \times 100$$

$$\text{Average residue weight} = \frac{1}{Z+N}$$

In view of these considerations it is suggested that, instead of per cent methoxyl by weight, the per cent galacturonide and the per cent esterification of the galacturonide chain be used in the characterization of pectin.

[2] The close agreement between the decrease in methyl ester groups and the increase in carboxyl groups observed on de-esterification of pectin indicate that, for the purpose of calculation, it is valid to consider that all the ester groups are methyl. In any case, if the araban or galactan occurred as ester groups, the decrease in the total carboxyl content would be negligible because of their high molecular weight.

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Inhibition of Growth of *Mycobacterium Tuberculosis* by a Mold Product—the Effect on Pathogenic Human Tubercle Bacilli [1,2]

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A mold product capable of inhibiting the *in vitro* growth of a rapidly proliferating nonpathogenic strain of *M. tuberculosis* was described in a previous report (*1*). The present study is concerned with the *in vitro* activity of this mold product against pathogenic human tubercle bacilli.

For convenience the material has been named, temporarily, mycocidin and a working unit of activity has been established. The strength of a mycocidin preparation is determined as follows: A standard culture is prepared consisting of a 5-day-old pellicle of the nonpathogenic strain of *M. tuberculosis* var. *hominis* (American type culture collection No. 607) on Long's synthetic liquid medium. The standard inoculum, which measures approximately 2 × 2 mm., is taken from the periphery of this pellicle. With some experience nearly similar fragments are usually obtainable. Test tubes of 1 in. in diameter containing 5 ml. of Long's synthetic medium are set up in series and mycocidin is added in varying concentrations. The final volume is made to 6 ml. The inoculum is floated

[1] From the Laboratories of the Hudson County Tuberculosis Hospital, Jersey City, New Jersey.

[2] The authors wish to acknowledge thankfully the accurate and capable assistance of Lyetta Yogman.

on the surface of the medium and the tubes are incubated at 37.5° C. for 5 days. The extent of growth of the inocula is recorded daily and compared with controls. A unit of mycocidin (M-unit) is defined as that amount of the preparation of the mold extract per milliliter of Long's synthetic medium which will completely inhibit the growth of the standard inoculum.

TABLE 1

Experiment number	Extract number	Concentration range (M-units per ml. Long's medium)	Strain of tubercle bacillus	Inoculum	Length of exposure of tubercle bacilli to mycocidin in Long's medium	Treated bacilli replanted and incubated for:	Minimal concentration necessary for complete inhibition (M-units per ml. Long's medium)
1	30	1.70 –2.66	RES	Large clumps	7 days	57 days	1.700
2	30 PD	0.13 –1.30	RES	Large clumps	7 days	44 days	0.515
3	30 PD	0.125–1.25	RES	1 mg. in suspension	7 days	50 days	0.625
4a.					18 hours	60 days	0.833
b.	34	0.20 –2.00	RES	1 mg. in suspension	3 days	58 days	0.625
c.					5 days	56 days	0.415
5	34	0.20 –2.00	CS	Large clumps	7 days	55 days	0.625

The human pathogenic tubercle bacilli employed in this experiment consisted of two strains which were isolated from patients with far-advanced pulmonary tuberculosis. The bacilli were grown on Petragnani's solid medium. Both strains are highly pathogenic for guinea pigs as evidenced by the fact that an inoculum of 0.0001 mg. of the bacilli suspended in normal saline and injected into the groin of guinea pigs produces diffuse miliary tuberculosis within 6 weeks.

The pathogenic human tubercle bacilli were treated with the mold product in the following manner: Tubes containing 5 ml. of Long's synthetic medium were set up in series. To one group mycocidin in varying concentrations was added. The other group served as a control. The final volume was made to 6 ml. Clumps or suspensions of tubercle bacilli were added to the tubes containing the extract as well as the controls. These were then incubated for periods varying from 18 hours to 7 days. The bacilli were centrifuged, washed with saline, recentrifuged, and resuspended in saline. The organisms were then tested for viability either by culturing on Petragnani's solid medium or by inoculation into guinea pigs.

The culture experiments are summarized in Table 1. As will be noted in Experiments 1, 2, and 5, the tubercle bacilli used consisted of clumps weighing from 1 to 2 mg. exposed to mycocidin in the Long's synthetic medium for a period of 7 days. The growth of the recultured bacilli was observed weekly, the final reading being made at periods of 44 to 57 days. Experiment 1 was a pilot experiment, and it soon became evident that more than the minimal amount of mycocidin necessary for complete inhibition of the organism had been used. In subsequent experiments, lower concentrations of mycocidin at graded intervals were employed. The minimal amount necessary for complete inhibition of growth was found to be approximately 0.515 M-unit per ml. of liquid medium for the RES strain and 0.625 for the CS strain. In Experiment 3, suspensions of organisms instead of clumps were employed. These suspensions were made by grinding a weighed quantity of bacilli in a mortar with saline containing several drops of bile. A final concentration of tubercle bacilli in suspension in saline of 1 gram per cent was made. One-tenth of a milliliter, equivalent to 1 mg. of bacilli, was added to each test tube containing the liquid medium with varying concentrations of mycocidin. It was found here, also, that the minimal concentration necessary for complete inhibition was 0.625 M-units per ml. In Experiment 4, the technique was similar to that of Experiment 3. However, the period of incubation was varied. Groups of tubes containing the extract and comparative controls were incubated for 18 hours, 3 days, and 5 days. As can be seen from the table, the minimal concentration necessary for complete inhibition was inversely proportional to the length of exposure of the tubercle bacilli to the mycocidin.

Cultures of the controls in all the preceding experiments showed active growth within 21 days, whereas those of the bacilli exposed to as little as

0.125 M-unit per ml. were still negative. At the time of the final reading all controls showed active, prolific growth, whereas all the cultures of the organisms treated with the minimal effective concentrations were negative. Bacilli that had been treated with less than minimal effective concentrations grew out slowly, the rate of growth being inversely proportional to the concentration of mycocidin to which they were exposed.

The animal experiments performed are summarized in Table 2. One milligram of the organisms of the RES strain in suspension was exposed to mycocidin in Long's liquid medium in the manner described above for periods of 18 hours, 3, 5, and 7 days at 37.5° C. The centrifuged and washed organisms were resuspended in saline and amounts equivalent to 0.1 and 0.5 mg. were injected into the groin of guinea pigs weighing 400–500 gms. Twenty-five animals were used. As can be seen from Table 2, when the organisms were exposed for a period of 3, 5, or 7 days to a concentration of 1 or 2 M-units per ml. and were injected into guinea pigs, the animals failed to develop tuberculosis. When killed 7 or 8 weeks later they were entirely negative on post-mortem examination. With lower concentrations and insufficient exposure, there was only partial inhibition of the bacilli as evidenced by the mild to moderate tuberculosis observed in the animals injected with these bacilli. All control animals showed far-advanced miliary tuberculosis.

Cultural studies and animal inoculations indicate that the mold extract, mycocidin, is both bacteriostatic and bactericidal for the human tubercle bacillus. After exposure to adequate concentrations of mycocidin, the bacilli fail to grow out when replanted on solid medium, and lose their ability to produce tuberculosis in guinea pigs. Since the treated bacilli are thoroughly washed, this cannot be due to adherent mycocidin. In concentration less than minimal, the bacteriostatic property of mycocidin is evidenced by the fact that when the bacilli are replanted on solid medium the rate of growth is slow in comparison to the controls and is inversely proportional to both the length of exposure and the quantity of mycocidin. It was found that 0.5 to 0.6 M-unit per ml. was necessary for bactericidal effect upon 1 mg. of the pathogenic strain of human tubercle bacilli. This would indicate that the pathogenic strains of human tubercle bacilli used in these experiments are about twice as sensitive to the effects of mycocidin as the nonpathogenic variety.

Further experiments are continuing on the isolation and purification of the active principle. The activity of mycocidin against other organisms is also being investigated.

TABLE 2

Extract number	Length of exposure of 1 mg. tubercle bacilli to mycocidin in Long's medium	Concentration of mycocidin in Long's medium (M-units per ml. Long's medium)	Number of guinea pigs	Mg. tubercle bacilli injected into guinea pig	Result
		0 (Controls)	4	0.1	All animals died within 28–35 days with diffuse miliary tuberculosis.
30 PD	7 days	1.0	4		One animal died after 6 days of pneumonia. Two were killed at the end of 30 and 51 days, respectively. One animal still alive at 127 days. No evidence of tuberculosis in any animal.
	18 hours	0	2		One animal died after 48 days. The other was killed after 54 days. Both animals had diffuse tuberculosis.
		0.2	2	0.5	One animal died after 46 days. The other was killed after 54 days. Both animals had diffuse tuberculosis involvement but less than controls.
		2.0	2		Both animals killed after 54 days—moderate tuberculosis present in one animal; other entirely negative.
	3 days	0	2		Both animals killed after 52 days—both extensive tuberculosis.
34		0.2	2	0.5	Both animals killed after 52 days—minimal tuberculosis in all viscera—early productive type lesions.
		2.0	2		Both animals killed after 52 days—both animals negative.
	5 days	0	1		Killed after 58 days—very extensive tuberculosis.
		0.2	2	0.5	Killed after 58 days. Liver and lungs normal. Focal tuberculosis in spleen and inguinal lymph node.
		2.0	2		Killed after 58 days—both animals negative.

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