

**Bactericidal Action of Pectin Containing Nickel.\***

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In a preliminary report on the bactericidal action of pectin, evidence was presented to show that the pectin used,† hereinafter referred to as pectin "A", in a pH range of 5.0 to 5.5 and after being subjected to certain thermal conditions, had a bactericidal action on *E. coli*.<sup>1</sup> At that time it was not understood to what this action was due. The possibility of a chemical contaminant had been immediately considered but assurance had been given by the producers‡ that all pectin was very closely checked for sulphur, lead, arsenic and other metals and that no impurity was then known which could account for the bactericidal action.

Further work was done on the action of unautoclaved pectin "A" on *E. coli*, the effect of autoclaved pectin "A" on a number of other organisms, the action of pectins from other producers§ and the action of 2 derivatives of pectin, namely pectic acid and methyl *d*-galacturonate. After this work was completed we were informed by the producers|| of pectin "A" that nickel was present in their product. The nickel is probably picked up during processing when the pectin solution is evaporated on a heated nickel drum. We have confirmed the presence of nickel in the pectin "A" and have tested the bactericidal activity of other samples of this pectin which were found to have varying amounts of nickel. The results of these tests, together with those of the work mentioned above, are presented here.

The method used for the bacterial counts was essentially the same as described in the first paper. *Escherichia coli* was again used and in addition *Staphylococcus aureus*, *Shigella paradysenteriae* Flexner, *Pseudomonas aeruginosa*, *Erwinia caratovor*a, a beta hemo-

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† Generously furnished by Sardik Laboratories, Inc., New York City.

<sup>1</sup> Haynes, E., Tompkins, C. A., Washburn, G., and Winters, M., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 839.

‡ Communication from Sardik Laboratories, Inc., New York City, April, 1936.

§ Kindly furnished by General Foods Corporation, Battle Creek, Michigan, and California Fruit Growers Exchange, Ontario, California.

|| Communication from Sardik Food Products Corporation, New York City, May, 1938.

lytic streptococcus and a diplostreptococcus from a case of ulcerative colitis. In the series in which the pectin was not subjected to heat, the pectin was added to the sterile broth, permitted to stand for 4 to 12 hours to insure thorough mixing and a fairly stable pH, necessary pH adjustments were then made and the rest of the procedure was carried out as given before. The nickel content<sup>¶</sup> (dimethylgloxime method) as well as the percent ash were determined on all pectins used.

The results given in Table I, show that when a 2% solution of pectin was used in heart infusion broth, pectin "A" was the only pectin tested which manifested bactericidal action. This action varied with the percentage of nickel present, no action being observed in a sample which contained as low as 0.16%. All other pectins, which were found to contain no nickel, as well as the pectic acid and methyl *d*-galacturonate, not only did not have an inhibitory action but permitted an abundant growth. The samples of pectin "A" having the higher percentages of nickel were found to have a markedly inhibitory action on *S. aureus*, *S. paradysenteriae* Flexner, *P. aeruginosa*, *E. caratovora* and the beta hemolytic streptococcus as well as *E. coli* but none on the diplostreptococcus. (Table II.) When not autoclaved, pectin "A" showed a slight inhibitory action which was somewhat variable but was never as marked as with the autoclaved solutions.

TABLE I.  
Typical Results of the Effect of 2% Solutions of Various Pectic Substances on Growth of *Escherichia coli* in Heart-infusion Broth.

Test solution	pH	Autoclaved or unautoclaved	Original count per cc	Count after 48 hours per cc	Nickel content %	Ash content %
Pectin "A"	5.0	A	2 x 10 <sup>5</sup>	0	0.78	1.57
" " "A"	5.1	U	3 x 10 <sup>5</sup>	2 x 10 <sup>6</sup>	0.78	1.57
" " "A"	5.3	A	1 x 10 <sup>5</sup>	0	0.22	1.2
" " "A"	5.0	A	8 x 10 <sup>4</sup>	9 x 10 <sup>8</sup>	0.16	1.5
" " "B"	5.3	U	9 x 10 <sup>4</sup>	1 x 10 <sup>9</sup>	0	0.59
" " "B"	5.3	A	9 x 10 <sup>4</sup>	8 x 10 <sup>7</sup>	0	0.59
" " "C"	5.0	U	5 x 10 <sup>4</sup>	2 x 10 <sup>9</sup>	0	2.07
" " "C"	5.4	A	2 x 10 <sup>5</sup>	1 x 10 <sup>9</sup>	0	2.07
" " "D"	5.3	A	8 x 10 <sup>4</sup>	8 x 10 <sup>8</sup>	0	3.14
" " "E"	5.3	A	3 x 10 <sup>5</sup>	4 x 10 <sup>6</sup>	0	4.67
" " "F"	5.2	A	1 x 10 <sup>5</sup>	7 x 10 <sup>7</sup>	0	3.95*
Methyl <i>d</i> -galacturonate	5.4	U	9 x 10 <sup>4</sup>	3 x 10 <sup>9</sup>	0	—
" " "	6.1	U	1 x 10 <sup>5</sup>	2 x 10 <sup>9</sup>	0	—
Pectic acid	6.2	A	2 x 10 <sup>5</sup>	4 x 10 <sup>8</sup>	0	—
" " "	6.4	U	2 x 10 <sup>5</sup>	2 x 10 <sup>8</sup>	0	—
Broth control	5.3		2 x 10 <sup>5</sup>	2 x 10 <sup>9</sup>	—	—
" " "	6.1		7 x 10 <sup>4</sup>	2 x 10 <sup>9</sup>	—	—

\*2.5% calcium present.

<sup>¶</sup> Grateful acknowledgment is due Dr. R. N. Harger for his generous assistance.

TABLE II.  
 Typical Results of Effect of 2% Pectin "A" on Growth of Various Organisms in Heart-infusion Broth.

	pH	Test organisms	Autoclaved or unautoclaved	Original count per cc	Count after 48 hours per cc
Pectin	5.4	<i>E. coli</i>	U	7 x 10 <sup>4</sup>	2 x 10 <sup>6</sup>
Control	5.4	"		1 x 10 <sup>5</sup>	1 x 10 <sup>9</sup>
Pectin	5.4	"	A	7 x 10 <sup>4</sup>	4 x 10
Control	5.4	"		7 x 10 <sup>4</sup>	7 x 10 <sup>7</sup>
Pectin	5.4	<i>S. aureus</i>	A	3 x 10 <sup>5</sup>	2 x 10 <sup>3</sup>
Control	5.4	"		3 x 10 <sup>5</sup>	6 x 10 <sup>8</sup>
Pectin	5.3	<i>S. paradysenteriae</i> Flexner	A	2 x 10 <sup>4</sup>	0
Control	5.3	" "		4 x 10 <sup>4</sup>	2 x 10 <sup>7</sup>
Pectin	5.5	Hemolytic streptococcus*	A	3 x 10 <sup>4</sup>	0
Control	5.5	" "		3 x 10 <sup>4</sup>	1 x 10 <sup>7</sup>
Pectin	5.2	<i>E. caratovorax</i> †	A	1 x 10 <sup>5</sup>	2 x 10 <sup>4</sup>
Control	5.2	"		9 x 10 <sup>4</sup>	2 x 10 <sup>7</sup>
Pectin	5.2	<i>P. aeruginosa</i>	A	1 x 10 <sup>5</sup>	2 x 10 <sup>4</sup>
Control	5.2	"		1 x 10 <sup>4</sup>	6 x 10 <sup>7</sup>
Pectin	5.4	Diplostreptococcus	A	1 x 10 <sup>5</sup>	8 x 10 <sup>7</sup>
Control	5.4	"		2 x 10 <sup>5</sup>	9 x 10 <sup>7</sup>

\*Final plates made at end of 24 hours' incubation.

†Tubes and plates incubated at room-temperature.

Since we could demonstrate bactericidal action only with pectin "A", which was found to be the only pectin tested containing nickel, and the action seemed to be in direct proportion to the amount of nickel present, it would seem that our reported bactericidal activity is a characteristic of this particular pectin containing nickel rather than of pectin *per se*. In what form the nickel is present, why its activity increases with heating, why the addition of sufficient NaOH to bring the pH to above 5.5 decreases the action and whether other metals in similar combination would have similar activity are matters for further investigation.

Preliminary work on the treatment of wounds with pectins containing no nickel indicates that though the bactericidal activity may be a slight contributing factor, there must be some other explanation for the total therapeutic value of pectin.

*Summary.* Pectin containing 0.22% or more nickel, after being subjected to certain thermal conditions and in a certain pH range, was found to have a slow bactericidal action when tested with *E. coli* and certain other bacteria. Other pectins which contained no nickel failed to demonstrate this activity.