

containing cell suspension was 5.0 cm. For the determination of the mortality, the number of survival cells was counted by plate culture.

It was found that the mortalities of organisms were higher when the cell suspension was taken in flat glass-bottomed container than in round bottom one, that the higher mortalities were again observed when the smaller amounts of suspension was employed, and that the concentration of cells had no effect upon the mortalities. Yeast cells in 20 ml suspension were killed perfectly in 25 minutes, however, more strength of the wave or treating time would be required to kill bacterial cells perfectly, since no remarkable effect was observed with bacteria in 30 minutes. Among the treated yeasts, retracted cells were detected and resolution of protoplasm from some cells were observed.

Secondly, the enzymatic activities (fermentation of glucose observed by Meissel tube) of the supernatant fluids of the living, pressed and dried yeast suspensions, obtained with the ultrasonic vibration for 25 minutes and centrifuged for 10 minutes, were compared, and it was found that living yeast revealed very much inferior to dried and pressed yeasts.

## 19. Studies on the Propionibacterium. (I)

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The isolation of propionibacterium from cow-milk has been tried. Many experiments were carried out with various culture media which contain yeast extract or pepton as nitrogen sources. Among them, we recognize the remarkable growth of the bacteria in the following two cultures. Namely, No. 3: yeast water containing 2% of Na-lactate as a carbon source, and No. 11: the mixture of the same amounts of skimmed milk and yeast water, added to 0.5% of CaCO<sub>3</sub>.

The amounts of volatile acids produced after two weeks' incubation in the described cultures (\*) and in yeast water containing 2% of lactic acid, 1% of pepton and 0.1% of NaCl (\*\*\*) were as follows.

No. 3	0.130 <sup>*</sup> N	0.151 <sup>***</sup> N
No. 11	0.018 N	0.196 N

The determination of Ducleaux Numbers of fermented liquors suggested the presence of propionic acid and acetic acid.

Plate culture containing 2% of glucose was tried to get colonies of propionibacteria. By repeating this method of isolation, four strains of bacteria were obtained, which form pin-head colonies and produce volatile acids, being non-motile and bearing no spores. Each shows an abundant growth in stab culture but a slight

growth on the surface, having catalase each reduces nitrate. One (3A41) is coccus and others are rodshaped, and the fermentation tests using the same medium as (\*\*) were as follows:

No. of strains	3A41	3A42	11N2	11N2
Lactec acid added (gr/100 cc)	2.45	2.45	2.45	2.45
Starting pH	7.2	7.2	7.2	7.2
Lactic acid remained ( % )	0.139	0.139	0.185	0.205
Final pH	5.8	5.6	6.2	6.2
Volatile acid produced ( % )	1.89	1.89	1.70	1.66
Propionic acid ( % )	1.29	1.43	1.18	1.32
Acetic acid ( " )	0.60	8.46	0.52	0.34

The fermentability of various sugars shows that strains 3A42, 11N2 and 11N3 are similar to *P. zeae*, *P. arabinosum* and *P. pentosaceum*, and the coccus is to *P. japonicum* respectively.

## 20. On the Action of Papain Enzyme. (IV)

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It has already been reported that rongalite (sodium formaldehydsulphoxylate ( $\text{CH}_2\text{OH}\cdot\text{OSONa}\cdot 2\text{H}_2\text{O}$ )) activates papain enzyme in gelatin decomposition.

In order to interpret the mechanism, rongalite and papain were estimated by the color reaction with fuchsin-sulphurous acid and by the BrCN-method.

It has been proved that the development of aldehyde reaction is more conspicuous in the case of the mixture of rongalite and papain solution than in the case of rongalite solution alone (See table).

Substance	O, I n $\text{Na}_2\text{S}_2\text{O}_3$ needed (in ccm)				
	hour of action (pH 5.0 at 37°C)	0	24	72	120
papain+rongalite		0.25	0.47	1.38	1.60
papain		0.10	0.10	0.15	0.20
rongalite		0.10	0.31	0.44	0.62

On the other hand, the papain enzyme solution mixed with rongalite and monojodo acetic acid has shown no digestability of gelatin. Furthermore, no decomposition of gelatin has been observed in the case of the papain solution treated with phenylhydrazine or p-nitrophenylhydrazine, even in the presence of rongalite.