compounds in treating experimental colon bacillus infections in mice, and would seem to be the drug of choice in treating *E. coli* tissue infections in human beings.

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Studies on Soft Curd Milk Prepared by the Enzyme Treatment Method.

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This is a preliminary report on the investigation of the characteristics of enzyme-treated milk.[1]

The enzyme treatment[1] is carried out by adding one part of pancreatic concentrate, which has a high tryptic value, to 10 to 15 thousand parts of cold raw whole cow milk and then pasteurizing the enzyme-milk mixture immediately in the usual manner. The milk proteins are altered by the pancreatic enzymes as the temperature of the milk rises during pasteurization.

Methods. Amino nitrogen was determined by the Van Slyke gasometric method. All other nitrogen determinations were made with the Kjeldahl method. The precipitin reaction was carried out according to the technic of Hektoen and Welker.[2] All control milk samples were prepared identically the same as the enzyme-treated milk samples and from the same milk without enzyme being added. The technic of the digestibility test which was found to be satisfactory is as follows:

Eight hundred ml of milk at 37°C is mixed with 80 ml of .5 N HCl containing .056% U.S.P. pepsin. The resulting pH is 4.8. Fifty ml portions are removed and filtered after 5 and 30 minutes' digestion at 37°C with mild agitation. The filtrates are set aside for total N analysis. The digest is then neutralized to pH 7.5 with NaOH and .112 g of U.S.P. trypsin is added. After 15 minutes of mild agitation at 37°C a third 50 ml portion is removed and adjusted to isoelectric pH 4.8 with HCl and then filtered to get the non-coagulable isoelectric protein filtrate. This is repeated every 20 minutes thereafter until a total digestion time of about 2½ hours

1 Conquest, V., Turner, A. W., and Reynolds, H. J., *J. Dairy Sci.*, 1938, **21**, 361.
2 Hektoen, Ludwig, and Welker, Wm. H., *J. Infect. Dis.*, 1924, **35**, 294.

TABLE I.
Comparison of the *In Vitro* Digestibility of Enzyme-Treated and Pasteurized Whole Cow Milk.

Time of digestion min.	g protein per 100 cc filtrate	
	Pasteurized whole milk	Enzyme-treated whole milk
5	.84	.96
30	.96	1.13
45	1.13	1.48
65	1.36	1.62
85	1.61	1.92
115	1.75	2.01
150	2.05	2.27

has elapsed. During the digestion, a pH level of 7.5 is maintained by the addition of NaOH. This test was used to study the digestibility of enzyme-treated milk.

Results. It was found that the enzyme-milk filtrates contained an average of 18.1% more protein than the control milk filtrates in 6 trials.

Table I shows the results of a typical digestibility test.

Enzyme-treated milk samples and their controls were made up to a 70% alcohol content, permitted to stand at room temperature over night and filtered. Total nitrogen was then determined on the filtrates to obtain the amount of alcohol-soluble protein in the milk. Table II shows that the enzyme treatment increases the amount of alcohol-soluble protein in whole milk.

No significant increase in the amino nitrogen content of enzyme-treated milk was observed. See Table II for the analytical results.

Proteose and peptone nitrogen of enzyme-treated milk and its control was measured by running total nitrogen on the trichloracetic acid filtrates of the milk. See Table II for the analytical data of this experiment.

These results do not show a significant increase in proteose nitrogen of enzyme-treated milk, but they do indicate a definite higher value.

Casein was prepared and purified from enzyme-treated skim milk

TABLE II.

Nitrogen fraction determined	g N per 100 ml milk		No. of trials
	Enzyme-treated whole milk	Regular pasteurized whole milk control	
Alcohol soluble protein	.174	.109	7
Proteose peptone	.0896	.0572	3
Amino acid	.019	.016	3

TABLE III.
The 70% Alcohol Solubility of Enzyme-Treated and Regular Pasteurized Skim Milk HCl and H_2SO_4 Caseins.

Type casein	g protein per 100 ml alcoholic filtrate		No. of trials
	Enzyme-treated casein	Pasteurized casein control	
HCl casein	.78	.44	5
H_2SO_4 casein	.079	.044	4

samples and their controls by precipitation with HCl or H_2SO_4 at 45°C. The curd was then washed 5 times with water, redissolved at pH 8.0 with NaOH, reprecipitated with acid, washed, dried at 45°C, and ground to 20 mesh size. Forty grams of the casein were then suspended in 250 ml of 70% alcohol for 3 days at room temperature. The undissolved casein particles were filtered from the alcohol and total nitrogen was determined on the alcoholic filtrate to estimate the solubility of the caseins in 70% alcohol. Table III shows the analytical data obtained in this experiment.

These results demonstrate the increased solubilities of enzyme-treated HCl and H_2SO_4 caseins in 70% alcohol.

Immunologic studies of 70% alcohol-soluble protein from raw skim milk showed that the immune sera obtained from rabbits immunized with this protein gave positive precipitin tests with enzyme-treated whole and skim milk, raw whole milk, raw whole milk rennet whey, pasteurized whole milk rennet whey, 70% alcohol-soluble protein from enzyme-treated skim milk, 70% alcohol-soluble protein from pasteurized skim milk, sweet cream dried buttermilk, and spray dried skim milk. Similarly these same immune sera gave negative precipitin tests with purified ox albumin, ox pseudoglobulin, lactoglobulin, lactalbumin and purified 70% alcohol-soluble protein prepared from pasteurized skim milk HCl casein.

Seventy percent alcohol-soluble protein from pasteurized skim milk HCl casein was used in an attempt to immunize rabbits. Excessive and repeated doses in 2 rabbits failed to produce sera giving positive results in the precipitin test.

The observed differences between pasteurized and enzyme-treated cow milk are as follows: Enzyme-treated milk contains more 70% alcohol-soluble protein and proteose peptone nitrogen, but only slightly more amino nitrogen than ordinary pasteurized milk. Casein prepared from enzyme-treated skim milk is more soluble in 70% alcohol than casein prepared from pasteurized skim milk.