



Title	PURIFICATION AND PROPERTIES OF A HEMOLYSIN PRODUCED BY HEMOLYTIC ESCHERICHIA COLI (O-139)
Author(s)	OHISHI, Iwao
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1. The percentage of high cell count milk (more than 500,000 cells per ml.), increased in proportion to the number of bacteria in milk.
2. No particular species of streptococci was isolated from the high cell count milk.
3. Sixty-seven strains of coagulase positive staphylococci were isolated from milk samples, 46 of them from high cell count milk.
4. Bacteriophage typing showed 62.5% of the 86 strains to be typable with phages of the international series and 67.5% with phages of NAKAGAWA's set. The strains isolated in Yakumo, Shizunai, and Obihiro were phagetyped primarily as group III with phages of international series, and A 1 and A 2 with NAKAGAWA's set, on the other hand, strains isolated from a herd in Sapporo were typed as group IV and A 1+A 2 respectively.
5. Of the total number of staphylococci strains isolated, 10 strains were resistant to 2 units of penicillin and 4 strains were resistant to 10 μ g streptomycin with the filter paper disk method.
6. The majority of the staphylococci produced beta lysin in combination with other lysins.

**PURIFICATION AND PROPERTIES
OF A HEMOLYSIN PRODUCED BY HEMOLYTIC
ESCHERICHIA COLI (O-139)**

Iwao OHISHI

*Department of Biochemistry,
Faculty of Veterinary Medicine,
Hokkaido University, Sapporo, Japan*

(Summary of Master's thesis written under direction of Dr. T. HAGA)

Attempts were made to purify and characterize a hemolysin produced by *E. coli* (O-139), which had been isolated from intestinal contents of a pig suffering from edema disease.

The original material used was culture filtrates of the organisms, which had been cultivated in acid-extract broth supplemented with 1 % peptone and 0.5% glucose.

The hemolysin was purified by adsorption and elution from calcium phosphate gel, fractionation with ammonium sulfate, and treatment with lead sub-acetate, followed by gel filtration with Sephadex G 50. The specific activity of the purified hemolysin increased by a factor of 1,300, with a 36% yield for activity.

The purified hemolysin was highly homogeneous electrophoretically and ultra-

centrifugally. It gave the usual color reactions for protein, e.g., biuret and TBP (Tetrabromophenolphthalein) reactions, and contained no detectable carbohydrate or phosphorus. The ultraviolet absorption spectrum of the purified hemolysin was characteristic for protein, with a maximum at 276 $m\mu$. The absorbancy at 280 $m\mu$ was found to be 1.1 for a 1 mg/ml solution.

These data suggest that the hemolysin is a protein-like substance.

The activity of the purified hemolysin was rapidly decreased by standing at room temperature or temperatures of 0° and -20°C.

The hemolytic reaction of the purified hemolysin had an optimum pH of 7.0 and was temperature-dependent below 40°C. It was found that the normal sera of cattle, pigs, sheep, and mice exerted an inhibitory effect on the hemolytic reaction.

The purified hemolysin was toxic to mice injected intravenously but not when injected intraperitoneally. The toxicity was closely correlated with hemolytic activity. The minimum lethal dose in mice was approximately 1,000 H.D.₅₀.

RELATIONSHIP OF FRUCTOSE CONCENTRATION AND BOVINE SEMEN CHARACTERISTICS OVER AN EXTENDED PERIOD

Kunitada SATÔ

*Department of Veterinary Obstetrics,
Faculty of Veterinary Medicine,
Hokkaido University, Sapporo, Japan*

(Summary of Master's thesis written under direction of Dr. T. ISHIKAWA)

1. Materials used were 103 semen samples obtained from 6 fertile Holstein bulls in an artificial insemination center. The semen was collected about every four days by the artificial vagina method. The semen samples used in this experiment were chosen at about 15-day intervals from May 1962 to July 1963.

2. A statistical analysis was made of eight semen characteristics using SPEAMAN's coefficient rank correlation and analysis of variance. None of these characteristics, i. e., volume and pH of semen, concentration of spermatozoa per milliliter semen, massive activity, individual motility, percentage of abnormal spermatozoa per 1,000, protoplasmic droplets per 200 spermatozoa, and fructose concentration per deciliter semen, showed any significant difference ($p < 0.05$).

3. There was no significant correlation between the fructose concentration and the concentration of spermatozoa or the volume of semen ($p < 0.05$).

4. The fructose content was estimated by the author's modification of MANN's