The present investigation was carried out to detect and study some of the metabolic products of enterococci with a view to correlate these, if possible, with the reported ability of this group of organisms to cause food poisoning.

1. Altogether 125 true enterococci were isolated from milk, milk powder and human and faecal matter and identified. These organisms were present in large numbers in all the materials examined. In milk and milk powder, these organisms were present in numbers more than those which would indicate direct faecal contamination. They were present at levels in which they could be considered to be acting as spoilage agents due to their known ability to grow and multiply fast in milk. Among the Sherman's criteria which are used for the identification of true enterococci, the tests for the growth in the presence of 6.5% sodium chloride and at 45°C were observed to be more reliable than other tests (growth at pH 9.6 and in 0.1% HB milk or survival at 60°C for 30 minutes) in correctly identifying an organism. Growth in the presence of 0.04% potassium tellurite and reduction of tetrazolium were useful in differentiating S. faecalis from S. faecium. Carbohydrate fermentation tests were, in general, not much helpful in the classification of organisms into species. Based on various biochemical tests employed, the 125 enterococci were tentatively classified as S. faecalis (38 nos.), S. faecalis var liquefaciens (14 nos.),
S. faecalis var zymogenes (25 nos.), S. faecium (34 nos.) and S. faecium var durans (14 nos.).

2. All the identified isolates were screened for their production of gelatinase, coagulase, DNase, haemolysin, lecithinase and a few strains for enterotoxin.

3. All the 14 cultures showing a gelatinase activity by the plate test were classified as S. faecalis var liquefaciens. The conditions for the production of gelatinase and some other characteristics of the enzyme were studied using S. faecalis var liquefaciens MM10, which produced maximum gelatinase.

4. Optimum conditions for gelatinase production was incubation at 37°C for 24 hrs. and Brain-Heart Infusion (BHI) broth adjusted to pH 7.5 supported the best growth and gelatinase elaboration by the test culture. Shake culture technique gave a slightly reduced yield of gelatinase than still culture.

5. Presence of gelatin upto 2% and ammonium sulphate upto 10% in BHI did not affect the gelatinase production by culture MM10. However, horse serum above 3.0% level and calcium chloride at 0.15% level in the medium suppressed the enzyme elaboration considerably.

6. Serial passage of the culture through mice tended to increase its virulence and ability to produce gelatinase. However, lethality of the culture appeared not to be due to gelatinase.

7. Enterococcal gelatinase was inactivated at 70°C within 3 minutes.
8. Out of 125 enterococci screened, 26 and 13 cultures gave positive reaction for slide and tube coagulase test respectively with 'Bacto' coagulase plasma. Among cultures with plasma clotting ability, 78.5% were *S. faecalis* var *liquefaciens*. All the *S. faecium* var *durans* cultures were negative for coagulase. The minimum time taken by the strains to show a positive slide and tube coagulase test was 25 seconds and 6 hours respectively. But there was no relationship between time taken for coagulation of plasma by slide and tube tests, since, *S. faecalis* var *liquefaciens* culture f2 which took longest time to give a positive reaction on slide clotted plasma quickest in tube. The experiments on growth conditions best suited for the production of coagulase were conducted using this particular strain.

9. *Streptococcus faecalis* var *liquefaciens* f2 coagulated plasma in tube within 2 hours when it was adapted to grow in a broth containing 0.5 to 1% citrate as the energy source. However, broth containing 2% sodium citrate inhibited coagulase production. Similarly, coagulase production decreased when citrate adapted culture was incubated with shaking. Tryptone Neopeptone Citrate (TNC) broth adjusted to pH 8.0 supported best growth and coagulase production by the test culture.

10. Bovine serum or bovine serum albumin fraction V failed to stimulate production of coagulase.

11. Plasma clotting action was observed to be associated mainly with the cells. Saline washings of the cells showed good plasma clotting activity suggesting that the coagulase was loosely bound to the surface of the cells.
12. In this present investigation, among 125 enterococci, 30 were found to possess an ability to elaborate a DNase enzyme. It appeared to be the first time such an observation is made. Majority of the DNase positive cultures were *S. faecalis*. However, DNase positive character was not observed to be the characteristics of any particular species among enterococci. Also DNase positive nature was not found to have any relationship with either coagulase, gelatinase or haemolysin production. Detailed studies on this enzyme was conducted with a *S. faecium* MP71 strain.

13. Enterococcal DNase was produced at a pH optimum between 6.4 and 6.7. It readily depolymerised calf thymus DNA but not DNA (Difco). The probable reason for this specificity is discussed.

14. A simple method for the assay of the enzyme, using Methyl Green DNA agar diffusion technique, has been developed.

15. In BHI, at an incubation temperature of 45°C, *S. faecium* MP71 elaborated considerably more quantity of DNase than when grown at 37 or 30°C.

16. Shaking the culture during incubation at 45°C depressed DNase production. The reason for this is considered to be poor growth of the organism under this condition and/or poor slime production.

17. Presence of calf thymus DNA in the growth medium (BHI) upto 20 mg/100 ml did not show any effect on DNase elaboration. However, 2% sodium bicarbonate and 0.5 to 4.0% dextrose in BHI decreased the
enzyme production by the culture. The reason for this decreased production is considered to be due to change in the pH of the medium outside the optimum range.

18. Streptococcus fascium MP71 showed moderate virulence to mice when injected intraperitoneally, but exhibited no increase in DNase producing ability on serial passage through mice.

19. Enterococcal DNase was observed to be remarkably thermostable. For its complete inactivation, the enzyme had to be held in a boiling water bath for 90 minutes. The similarity of this enzyme in this respect to that of pathogenic staphylococci, and thus, the possible significance of this character in enterococci is discussed.

20. None of the enterococci isolated was capable of producing lecithinase. Production of haemolysin was an unstable character among these organisms. A free haemolysin in enterococcal broth cultures was found to be difficult to assay.

21. An ability to induce accumulation of fluid inside the ligated loops of rabbit ileum by few enterococcal cultures was demonstrated for the first time. The metabolic product responsible for the production of this response is considered to be an enterotoxin.

22. The macroscopic appearance of a positive loop resembled that reportedly produced by enteropathogenic V. cholerae, E. coli, B. cereus and S. aureus. The fluid inside the loop was thick, straw yellow in colour with no foetid smell.
23. The time required for the pronounced fluid accumulation to take place varied from 6 to 9 hours. The possible maximum response could not be studied as the experimental animals usually did not survive more than 10 hours after challenge.

24. *S. faecium* MP71 gave a loop fluid length ratio of 1.5 within 24 hours when the culture was incubated in BHI at 45°C. Culture incubated at 37°C produced a maximum loop fluid length ratio of only 0.8 by 48 hours of incubation. No toxin could be detected in the culture supernatant fluid after 96 hours. The possible reasons for increased production at higher temperature of incubation are discussed.

25. A relationship between enterotoxin and DNase production among enterococci was observed. Majority of enterotoxigenic enterococci were either DNase positive. Production of both was more at 45°C than at 37°C.

26. Enterotoxigenicity was not observed to be associated with any species among enterococci. Both *S. faecalis* and *S. faecium* and their varieties were found to be capable of elaborating this toxin.

27. The toxin was heat labile, non-dialysable and inactivated by trypsin.

28. This study thus revealed that certain strains of enterococci elaborate an enterotoxin that gives a positive rabbit ileal loop response indicating the probable role these organisms are capable of playing in human food poisoning outbreaks. Most of these strains may be identified by their thermostable DNase positive character. Certain limitations observed in this investigation are discussed.