Production, purification and properties of lipase from *Streptococcus faecalis*
DISCUSSION

I. DISTRIBUTION OF LIPASE ACTIVITY

From the relationship between the growth and lipase production by *Streptococcus faecalis*, it is evident that a gradual increase in lipase activity was noted throughout the logarithmic growth phase and maximum yield of lipase was obtained after 24 hr incubation of the culture in the late stationary phase (Fig. 1). A substantial decline in lipase production was observed in 48 hr (Table 1). This decreased enzyme production after 24 hr could perhaps be due to lack of fresh synthesis of lipase during the phase of decline, thereby resulting in partial inactivation of the enzyme. However, Mates and Sudakowitz (1973) obtained maximum amount of extracellular lipase in *Staphylococcus aureus* after 48 hr of incubation of culture at 37°C.

Lipase production by *S. faecalis* was noted to be extracellular. This finding is in agreement with that of Fryer et al. (1967), who demonstrated that lipases produced by lactic acid bacteria are extracellular in nature. According to Davies (1963), maximum yield of extracellular enzyme was possible when the growth of the organism ceased, provided the medium did not become deficient in ingredients which are specifically required for
enzyme synthesis. *S. faecalis* lipase satisfied the
criteria laid down by Pollock (1962) for extracellular
enzymes, as the enzyme appeared during the logarithmic
phase of growth of the culture, when the cells were not
prone to lysis. Further, the enzyme was not found inside
the cells. The extracellular nature of lipase produced
from *S. faecalis* has been confirmed further by the
presence of negligible enzyme activity in intracellular
portion of cell as well as in cell debris during the
logarithmic and late stationary phases of growth.

II. FACTORS AFFECTING LIPASE PRODUCTION

Highest yield of lipase was obtained in 24 hr at
30°C as compared to 22, 37 and 45°C and enzyme production
declined considerably in 96 hr (Table 2). These results
are in agreement with the observations of Nadehra and
Harmon (1965a) on *Staphylococcus aureus* which produced
maximum amount of lipase at 30°C as compared to 15 and
22°C. Results of the present study are contradictory to
earlier reports on maximum lipase production at 21°C by
many lipolytic bacteria (Harmon and Nelson, 1955, 1957 and
Khan et al., 1967). In the present study, an attempt has
been made to obtain maximum amount of lipase in a shorter
time and for this purpose, temperature of incubation at
30°C for 24 hr was found satisfactory. Highest yield of enzyme obtained at this temperature may be attributed to accelerated metabolic activity of the organism and there is also a tendency towards shorter log phase. The decrease in the yield of lipase noted at a still higher temperature like 45°C, may be due to partial denaturation of the enzyme. However, Liu et al. (1972) reported that 45°C was optimum for the production of lipase by Humicola lanuginosa.

S. faecalis was capable of producing considerable amount of lipase over a wide pH range from 6.0 to 8.0 (Fig. 2), although maximum lipase production occurred at pH 7.5. It may be noted in this connection, that hydrogen ion concentration influenced both the growth and enzyme production by this organism. Mates and Sudakevitz (1973) reported a similar pH requirement for lipase production by Staphylococcus aureus. An initial pH > 7.0 has been recorded for maximum lipase production by Penicillium roqueforti (Niki et al., 1966), Micrococcus freudenreichii (Lawrence et al., 1967a) and Staphylococcus aureus (Vadehra, 1974).

Out of various carbohydrates that were tested for lipase production by glucose (1%) promoted maximum growth and enzyme production (Fig. 3).
Further increase in glucose concentration, depressed the growth as well as lipase yield. Although mannitol also stimulated enzyme activity, the results were not as significant as in case of glucose (Table 3). The other four carbohydrates such as maltose, galactose, sucrose and arabinose invariably reduced enzyme production. The results of the present study are contrary to the findings of Iwai and Tsujisaka (1974) who did not find significant differences in lipase yield when different carbohydrates were tested.

Addition of glucose to the basal medium was essential for both the growth and lipase production by *S. faecalis*, since omission of the above carbohydrate source resulted in very little growth and enzyme yield. These findings are in accordance with those of Alford and Pierce (1963) who failed to observe lipase synthesis in *Pseudomonas fragi* in absence of glucose. Nelson (1953) reported enhanced lipase synthesis in *Geotrichum candidum* when the organism was grown in a medium containing 1% carbohydrate. Increased yield of lipase in *Pseudomonas aeruginosa* was obtained by inclusion of 0.2% glucose in the nutrient medium, while further addition of glucose resulted in decreased lipase production (Nadkarni, 1971). Retardation in lipase production by carbohydrate sources
other than glucose as noted in the present study, may have to be explained in the light of some observations made by Mates and Sudakevitz (1973) on lipase synthesis by \textit{Staphylococcus aureus}. According to the above workers, inhibition of lipase production by some sugars depended upon the ability of bacteria to metabolize carbohydrates or due to production of certain lipids during the metabolism of these carbohydrates. It can further be presumed that addition of more than 1% glucose may divert the excess glucose towards the synthesis of lipids or substances that are known to retard enzyme production.

Peptone stimulated both the growth and lipase production by \textit{S. faecalis}. As compared to peptone, proteose-peptone and tryptone were less effective (Table 4). In this respect, \textit{S. faecalis} is similar to \textit{Pseudomonas fragi}, since stimulation in lipase production was observed in this organism, in presence of peptone and proteose peptone (Nashif and Nelson, 1953b). The yield of lipase by \textit{S. faecalis} was maximum when 2% peptone was used in the basal medium (Table 5). Lawrence \textit{et al.} (1967a) also found that both the growth and lipase yield by \textit{Pseudomonas fragi} were stimulated at 2% level of peptone in the growth medium. While studies
on many lactic acid bacteria, Umemoto (1969) observed that peptone was effective at a lower level of 1.5%. Mates and Sudakevitz (1973) reported lipase production by *Staphylococcus aureus* with 1% peptone, but not at 0.5% level, while maximum synthesis of enzyme occurred at 2% level.

*S. faecalis* is known to grow even at higher concentration of NaCl. The results of the present study with *S. faecalis* 272 have shown that although growth of the organism was unaffected, lipase production was inhibited by more than 0.5% NaCl (Table 6, Fig. 4). These results are comparable to similar findings by Mates and Sudakevitz (1973) on *Staphylococcus aureus* which, although grew well in presence of high concentration of NaCl, produced lesser amounts of lipase at a concentration of NaCl above 0.2%. Inhibition of lipase synthesis at higher NaCl concentrations has, however, been reported for other microbial lipases such as Bacillus, *Staphylococcus*, *Micrococcus*, and *Proteus* (Scheibner, 1973). On the other hand, Sorhaug (1974) has shown that production of lipase by *Brevibacterium linens* was independent of salt concentration.

Addition of calcium, manganese and magnesium chlorides to the basal medium enhanced the growth as well as yield of lipase by *S. faecalis* (Table 7). Although the above salts are not associated with buffering
action, increase in lipase production by \textit{S. faecalis} may be attributed to the stimulatory effect of calcium, manganese and magnesium ions on the biosynthesis of lipase. The essentiality of calcium salts for lipase synthesis has also been definitely demonstrated in \textit{Pseudomonas aeruginosa}. This organism failed to synthesize lipase in decalcified broth medium (Sierra, 1957).

Amongst inorganic nitrogen compounds which were tested for lipase production, ammonium sulphate was more effective in enhancing growth and lipase production, as compared to ammonium acetate and ammonium chloride (Table 8); potassium salt depressed lipase yield. Thibodeau and Macy (1942) and Eitenmiller \textit{et al.} (1970) reported sodium nitrate as a poor inorganic nitrogen source for the growth and lipase production by \textit{Penicillium roqueforti}.

\textit{S. faecalis} required some of the essential amino acids for growth and lipase production. The omission of some of the amino acids like arginine, glutamic acid, histidine, isoleucine, leucine, methionine, threonine, tryptophan and valine from the synthetic medium resulted in marked decline in growth as well as lipase production (Table 9). Although Greenhut \textit{et al.} (1946) had reported earlier that 13 amino acids were essential
for the growth of *S. faecalis*, while amino acids like cysteine, serine and lysine which were found essential by the above workers, were not essential in the present study.

Lipase produced by *S. faecalis* was constitutive and was synthesized in larger quantities in growth media devoid of substrates. However, maximum amount of lipase was produced by this organism in presence of 0.5% tributyrin followed by tricaprin and tricaprylin (Table 10, 11). The results of the present study have clearly indicated that *S. faecalis* utilizes triglycerides containing lower chain saturated fatty acids for growth as well as lipase production. Triglycerides with long chain unsaturated fatty acids were not properly utilized by the organism, thereby retarding growth and lipase synthesis. Lower chain saturated fatty acids like butyric and caproic acids stimulated lipase production by *S. faecalis* (Table 12), since these fatty acids were utilized in preference to other long chain saturated fatty acids. Smith and Alford (1966) also indicated that inhibition of lipase production by lard in *Pseudomonas fragi* was presumably due to accumulation of unsaturated free fatty acids following hydrolysis of fat. The inhibitory
effect of unsaturated fatty acids on the growth and lipase activity (Table 12) has also been attributed to the bacteriostatic effect of the above compounds (Strong and Carpenter, 1942). According to Aden (1941), unsaturated fatty acids form a monolayer around bacteria and exert their effect in at least three different ways either by changing the permeability of adjacent surfaces or by exerting some chemical influence or by altering the surface tension, thereby interfering with cell division. Due to greater number of double bonds present in unsaturated fatty acids, a larger number of monolayers are expected to be formed in them than in saturated fatty acids. Thus, greater inhibition of growth occurs in presence of highly unsaturated fatty acids as compared to lower unsaturated fatty acids. The above hypothesis may explain the reduced growth and retarded lipase production by \textit{S. faecalis} in presence of triglycerides containing unsaturated fatty acids.

Lipase production by \textit{S. faecalis} did not appear to be adaptive in true sense of the term and in this respect, \textit{S. faecalis} lipase resembled \textit{Pseudomonas frari} lipase (Nashif and Nelson, 1953b), in that some of the triglycerides were slightly inhibitory, while others were stimulatory to lipase synthesis. According to Davies (1963), an enzyme can be categorized as either constitutive
or adaptive. Constitutive enzyme formation is not a completely fixed property but is known to vary depending upon the conditions of the growth of organism.

Several workers have confirmed the adaptive nature of microbial lipases, since these are known to be stimulated by lipid substrates in the growth medium (Cutchins et al., 1962; Yoshida et al., 1968; Ota et al., 1968 and Formisano et al., 1975). The inhibitory action of lipids on lipase synthesis has also been recorded in some microorganisms like *Pseudomonas fragi* (Smith and Alford, 1966), *Penicillium roqueforti* (Eitenmiller et al., 1970) and *Staphylococcus aureus* (Mates and Sudakevitz, 1973).

III. PURIFICATION OF LIPASE

*Streptococcus faecalis* - 272 lipase was purified by carrying out a combination of purification procedures involving ammonium sulphate precipitation, gel filtration, acetone fractionation and column chromatography (Table 13, Fig. 5 to 8).

The precipitation of the crude enzyme with ammonium sulphate at 60% saturation, resulted in 85% recovery of enzyme activity with 12.4 fold purification. A similar procedure with slight modifications of saturation levels
of ammonium sulphate has been adopted for purification of lipases to varying degrees from many microorganisms (*Mycoplasmá gallisepticum*, Rotten and Razin, 1964; *Pseudomonas fragi*, Kencher and Alford, 1967 and *Propionibacterium shermanii*, Oterholm *et al.*, 1970).

Passage of enzyme through Sephadex G-25 purified it to 14 fold with 70% recovery of enzyme activity (Fig. 5). By adopting the above technique, Fukumoto *et al.* (1964) and Oterholm *et al.* (1970) achieved only 7.8 and 2.2 fold purification of *Rhizopus delemar* and *Propionibacterium shermanii* lipases, respectively.

The pooled enzyme fractions from Sephadex G-25, on precipitation with 60% v/v acetone, resulted in a recovery of 58% lipase activity, with 23.2 fold purification (Table 13). Fukumoto *et al.* (1963) also succeeded in purifying *Aspergillus niger* lipase to 14 fold after acetone fractionation. However, by adopting the above procedure, Lu and Liska (1969) achieved 48 fold purification of *Pseudomonas fragi* lipase.

Further purification of the partially purified *S. faecalis* lipase through Sephadex G-75 column, resulted in 70 fold purification with 50% recovery of enzyme
activity (Table 13 and Fig. 6). Results of the present study are comparable to that of Lu and Liska (1969), who purified \textit{Pseudomonas fragi} lipase to 74.5 fold by Sephadex gel filtration.

The pooled fractions collected after Sephadex G-75 filtration when passed through DEAE cellulose column, purified the enzyme to 140 fold (Table 13, Fig. 7) with 30\% recovery of enzyme activity. The technique of DEAE cellulose column chromatography has also been employed for the purification of lipases of \textit{Staphylococcus aureus} (O'Leary and Weld, 1964) and \textit{Pseudomonas fragi} (Lu and Liska, 1969). The results of the present work are in accordance with those of Oterholm et al. (1970) who achieved 141 fold purification of intracellular lipase from \textit{Propionibacterium shermanii} by DEAE cellulose chromatography.

Pooled fractions obtained from DEAE cellulose were further purified through DEAE Sephadex A-50 yielding 20\% recovery of enzyme activity and 280 fold purification was achieved (Table 13, Fig. 8). A similar purification procedure had been adopted by Rottem and Razin (1964) for \textit{Mycoplasma Gallisepticum} and Mencher and Alford (1967) for \textit{Pseudomonas fragi} lipases. Ota et al. (1970) also purified lipase from \textit{Candida paraparapolytica} using the above technique.
But it is interesting to note that in the present study, a greater degree of purification of *S. faecalis* lipase to the extent of 280 fold has been achieved than with many other microbial lipases.

IV. HOMOGENEITY OF LIPASE

Purified *S. faecalis* lipase exhibited single band moving towards the anode by SDS polyacrylamide electrophoresis (Fig. 9), thereby indicating homogeneity of the enzyme preparation. In this respect, *S. faecalis* lipase resembled *Chromobacterium* and *Geotrichum candidum* lipases (Sugiura et al., 1974 and Jensen, 1974).

Ultracentrifugal analysis of purified *S. faecalis* lipase confirmed that this enzyme has essentially a single homogeneous protein as evidenced by the appearance of single symmetric peak (Fig. 10), thus resembling other microbial lipases (like *Torulopsis* (Hotai et al., 1966), *Penicillium crustosum* (Oj et al., 1967) and *Rhizopus* species (Oj et al., 1969).

The homogeneity of *S. faecalis* lipase has also been further confirmed by isoelectric focussing technique (Fig. 11, 12). The isoelectric point of the purified enzyme was found to be at pH 3.6. A wide variation in
the isoelectric points of different microbial lipases has been reported in literature. Imamura and Kataoka (1966) observed that *Penicillium roqueforti* lipase had two different isoelectric points, one at pH 8.0 and the other at 4.0. *Mucor* lipase also exhibited two isoelectric points, one at pH 9.7 and the other at 10.2 (Nagaoka and Yamada, 1973). The results of the present studies on *S. faecalis* lipase showing one isoelectric point are comparable to the findings of Motai et al. (1966), Tsujisaka et al. (1973) and Sugiura et al. (1974) on other purified microbial lipases like *Torulopsis, Geotrichum candidum* and *Chromobacterium*, which exhibited isoelectric points at pH 2.0, 4.33 and 4.7, respectively.

V. AMINO ACID ANALYSIS

Results of amino acid analysis of purified *S. faecalis* lipase (Table 14) indicated that among acidic amino acids, glutamic acid was present in largest amount, while amongst basic amino acids, histidine was detected in smallest quantities. The enzyme contained very little amount of cysteine among the sulphur containing amino acids. Amino acids composition of *Geotrichum candidum* showed the absence of sulphur containing amino acids (Tsujisaka et al., 1973).
VI. MOLECULAR WEIGHT

The molecular weight of *S. faecalis* lipase as determined by gel filtration technique was 20,000 (Fig. 13). The results of gel filtration chromatography also suggested that this lipase possessed a single homogeneous component. The molecular weight of *S. faecalis* lipase was thus comparable to *Micrococcus* and *Pseudomonas* lipases (mol. wt. 25,000, Lawrence et al., 1967a). These results also showed that bacterial exoenzymes are usually small proteins (Pollock, 1962).

Considerable variations in molecular weights of lipases from microorganisms have been found in literature (*Penicillium crustosum*, mol. wt. 29,000 and 32,000, Qi et al., 1967; *Candida mycoderma* and *Debaryomyces klockeri*, mol. wt. 30,000 - 35,000 and 12,000 - 21,000 respectively; Hozono and Tokita, 1970; *Humicola lanuginosa* S-38, mol. wt., 27,500, Liu et al., 1973c; *Staphylococcus aureus*, mol. wt. 100,000, Vadehra, 1974; *Mucor*, F-3A and F-3B, mol. wt. 25,400 and 29,000, Nagaoka and Yamada, 1973 and *Geotrichum candidum*, mol. wt., 53,000-55,000, Tsujisaka et al., 1973).

VII. PROPERTIES OF PURIFIED *S. FAECALIS* LIPASE

Michaelis constant for purified *S. faecalis* lipase has been found to be 5.0 x 10^-3 M at pH 7.5 and at 40°C,
using tributyrin as substrate (Table 15, Fig. 14). The Km of purified Propionibacterium shermanii lipase has been reported as $2.0 \times 10^{-3}$ M at 35°C (pH 7.2) with tributyrin substrate (Oterholm et al., 1970). Variations in Km as reported above may be ascribed to differences in the source of the enzyme.

A linear relationship was noted between the activity of S. faecalis lipase and enzyme concentration (Fig. 15). These results are comparable to similar observations on purified lipases of Propionibacterium shermanii (Oterholm et al., 1970) and of Pseudomonas aeruginosa (Nadkarni, 1971).

Amongst several emulsifiers that were examined in this investigation, gum acacia formed the best emulsion and promoted highest lipase activity as compared to other emulsifying agents (Table 16). Oi et al. (1969) suggested that out of the different emulsifiers tested by the above workers, polyvinyl alcohol was the best for lipase activity in Rhizopus species. The use of other emulsifying agents like agar-agar, pectin, lecithin for determining lipase activity of S. faecalis showed that the results were comparable with the earlier findings of Oi et al. (1969).
The hydrolytic action of \textit{S. faecalis} lipase was dependant upon the type of substrate, although the enzyme was capable of hydrolysing both synthetic and natural triglycerides (Table 17). Amongst synthetic triglycerides, tributyrin was hydrolysed more readily than other simple synthetic triglycerides and in this respect, \textit{S. faecalis} lipase resembled several other microbial lipases like those of \textit{Penicillium roqueforti} (Shipe, 1951; Morris and Jezeski, 1953 and Eitenmiller \textit{et al.}, 1970), \textit{Staphylococcus aureus} (Shah and Wilson, 1965), \textit{L. casei}, \textit{L. plantarum}, \textit{L. helveticus}, \textit{L. acidophillus} and \textit{S. diacetilactis} (Sato \textit{et al.}, 1967 and Umemoto \textit{et al.}, 1968) and \textit{Chromobacterium} (Sugiura \textit{et al.}, 1974).

\textit{S. faecalis} lipase hydrolysed butter oil to a lesser degree than tributyrin. Such a preferential hydrolysis of tributyrin has also been reported in mammalian tissues (Wills, 1961). Triglycerides with a chain length of four to eight carbon atoms were hydrolysed more rapidly by \textit{Penicillium roqueforti} lipase (Shipe, 1951).

Since natural oils were not split off more readily, it may be inferred that \textit{S. faecalis} lipase had less preference for lipids with long chain unsaturated fatty acids. According to Clément and Clément-Champougny (1954), fat with low
degree of unsaturation were hydrolysed by lipase more readily than unsaturated glycerides.

The apparent specificity of \textit{S. faecalis} lipase for short chain saturated fatty acids in the simple triglycerides, may be due to reactivity with which these substrates form emulsion at 37°C. Further the same rate of hydrolysis may not be achieved presumably due to marked differences in the molecular weight as well as physical state of synthetic and natural triglycerides.

The effect of pH on the activity of purified \textit{S. faecalis} lipase has been illustrated in Fig. 16. The optimum pH for enzyme activity was 7.5 and any variation in optimum pH level, was accompanied by an inhibition of enzyme activity. Umemoto \textit{et al}. (1968) and Carini (1969) reported similar results in regard to optimum pH range of 6.0 to 8.0 for lipolytic activity amongst lactic acid bacteria. There are, however, some reports on microbial lipases like \textit{Aspergillus niger}, \textit{Rhizopus delemar}, \textit{Mucor pusillus} and \textit{Candida muscorum}, which showed maximum lipase activity at pH below 7.0 (Fukumoto \textit{et al}. , 1963, 1964; Somkuti \textit{et al}. , 1969 and Hosono \textit{et al}. , 1973). Higher pH optima above 7.0 for lipase activity in microorganisms like \textit{Serratia marcesens}, \textit{Achromobacter lipolyticum}, \textit{Micrococcus} species, \textit{Pseudomonas fragi}, \textit{Penicillium cassinicum} and
Mucor have also been well documented by other workers (Gorbach et al., 1965; Shahani et al., 1964; Lawrence et al., 1967a; Mencher and Alford, 1967; Lamberet and Lenoir, 1972 and Nagaoka and Yamada, 1973).

The optimum reaction temperature for purified S. faecalis was 40°C (Fig. 17). Any further increase in reaction temperature resulted in inactivation of the enzyme. The results of the present study corroborate the findings of Umemoto et al. (1968) in regard to the optimum reaction temperature range of 30 to 40°C required for activity of lipases of lactic acid bacteria. Both higher (Motai et al., 1966; Oterholm et al., 1970 and Liu et al., 1972) and lower temperature optima for activity of microbial lipases have been reported in literature (Carini, 1969; Hosono and Tokita, 1970; Driessen and Stadhouders, 1971; Lamberet and Lenoir, 1972; Chander et al., 1973 and Vadehra, 1974). It is presumed that emulsified fat globules may be in a more dispersed state in the temperature ranges mentioned above and optimum temperature for lipase activity usually falls within this range. Since lipolysis is an enzymatic reaction at the interface between fat globules and aqueous phase, the stability of fat emulsion is said to vary with different reaction temperatures (Summer, 1954). The optimum reaction temperature for lipolytic action may,
therefore, be considered an important factor in controlling the rate of this reaction. According to Khan et al. (1967), the physical state of the substrate may not be conducive to interaction with the active site of the enzyme, when the reaction temperature is less than the optimum.

From the results on the storage of purified *S. faecalis* lipase at different temperatures (Fig. 18), it may be noted that loss of activity was pronounced on storage of enzyme at 37°C for 7 days, while 95% enzyme activity was retained when the enzyme was stored at -18°C for 30 days. These observations are in agreement with those of Somkuti et al. (1969) on *Mucor pusillus* lipase which retained its activity at -10°C for 30 days. Troller and Bozeman (1970) also found no loss in lipase activity on storage of *Staphylococcus aureus* lipase for 4 weeks under frozen condition.

*S. faecalis* lipase was completely inactivated at 90°C for 10 min, while at 80°C for 10 min, the inhibition of enzyme activity was nearly 90% (Fig. 19). According to the report of Driessen and Stadhouders (1971), *Alcaligenes viscolactis* lipase lost 92% of its activity at 84°C for 10 sec and was completely inactivated at 90°C for 10 sec. *S. faecalis* lipase differed markedly from *Achromobacter*.
lipolyticum lipase, since the latter was more heat stable and was inactivated at 99°C within 40 min (Khan et al., 1967). Although the mechanism of thermostability of lipase is not clearly understood, Manning et al. (1961) attributed the above property to the structure of enzyme itself. Two explanations have been offered in regard to the structure of thermostable enzyme. According to first theory, the enzyme exists as a randomly coiled structure, while the second explanation envisages the existence of the enzyme as a rigid structure. During the process of inactivation, the enzyme is said to undergo unfolding and uncoiling. It is possible that purified \textit{S. faecalis} lipase may have a rigid or randomly coiled structure in view of its thermostability, but further studies may substantiate this conjecture. This thermostability forms the outstanding feature of this enzyme and emphasizes the necessity of avoiding the post pasteurisation contamination of dairy products with \textit{S. faecalis}.

In regard to the effect of pH on the stability of purified \textit{S. faecalis} lipase, complete retention of enzyme activity was noted between pH 6.0 to 8.0 (Fig. 20). The present results are in partial agreement with the findings of Mencher and Alford (1967) and Oj et al. (1967) on \textit{P. fragil} and \textit{Penicillium crustosum} lipases, respectively.
These two lipases exhibited stability at pH levels between 6.0 – 9.0. Other microbial lipases such as those obtained from *Torulopsis* (Mochai *et al.*, 1966), *Candida paralipolytica* (Ota *et al.*, 1970) and *Chromobacterium* (Sugiura *et al.*, 1974) showed stability at a higher pH range of 3.0 to 9.0. In contrast to above reports, increase in pH above 8.5 or fall in pH below 5.0, resulted in inactivation of *S. faecalis* lipase.

Data on the effect of bile salt on the activity of *S. faecalis* lipase indicated that bile salt at lower concentration (0.2%), stimulated maximum enzyme activity. Enhanced activity by bile salts has also been reported for other microbial lipases such as those from *Leptospira* (Patel *et al.*, 1964), *Candida paralipolytica* (Ota *et al.*, 1970), *Mucor* (Nagaoka and Yamada, 1973) and *Chromobacterium* (Sugiura *et al.*, 1974). Wills (1965) suggested that the stimulatory action of bile salt on the activity of mammalian lipase may be due to its surface active property which promoted the rate of hydrolysis by increasing the interfacial area of fat aqueous phase. It is possible that the stimulatory action of bile salt on *S. faecalis* lipase may be due to better alignment of the enzyme to the substrate molecule, thereby accelerating the hydrolysis of triglycerides.
Several metallic salts like magnesium, cobalt, sodium ferrous and manganese stimulated the activity of purified *S. faecalis* lipase (Table 13). These observations are similar to those of Khan et al. (1967) on enhanced activity of *Achromobacter* lipase by magnesium and sodium ions. Similarly, Eitenmiller et al. (1970) showed that *Penicillium roqueforti* lipase was activated by manganese and magnesium ions. Ota et al. (1970) also found stimulation in the activity of *Candida paralinolytica* lipase by sodium ions and this was attributed to the high ionic strength of sodium salt. The results on inhibitory effect of copper salt on the activity of *S. faecalis* lipase are in agreement with reports on lipases produced from microorganisms such as *Torulopsis* (Notai et al., 1966) and *Rhizopus* species (Oi et al., 1969). The inhibition of *S. faecalis* lipase activity by zinc salt is comparable to similar findings on other microbial lipases (*Aspergillus niger*, Iwai et al., 1964b; *Penicillium roqueforti*, Eitenmiller et al., 1970 and *Lactobacillus brevis*, Chander et al., 1973).

The rate of hydrolysis of butter oil, by purified *S. faecalis* lipase was appreciably stimulated by calcium ions (Fig. 22). This observation corroborates the reports of some workers (Iwai et al., 1964b; Wills, 1965; Oi et al., 1969, Chander et al., 1973 and Sugiura et al., 1974), while it differs from the results of other workers (Khan
et al., 1967 and Eitenmiller et al., 1970) who showed that calcium ions had no effect on lipase activity. The mechanism of action of calcium ions on lipase activity has been explained by several workers. Ota and Yamada (1966) considered that the oil-water interface was important for elucidation of the activation mechanism by calcium ions. The above workers found that the sigmoidal activation brought about by calcium ions was of first order and a cationic surfactant acted as a competitive inhibitor against calcium ions. It was further indicated by above investigators that the enzyme required one calcium ion per active centre which may be connected with unknown negatively charged groups. In a later communication, Ota and Yamada (1967) reported that calcium ions promoted the alignment of enzyme on the substrate molecule. Oi et al. (1967) were of the opinion that calcium ions enhanced the lipase activity by stabilizing the active configuration of the enzyme. According to Liu et al. (1973b), the primary role of calcium ions is to remove fatty acids formed during hydrolysis as insoluble calcium soaps, thereby changing the interfacial substrate-water relationship so as to make it favourable for enzyme action.

Some specific SH reagents such as $\beta$-hydroxymercuribenzoate, iodoacetate and mercuric chloride brought about
little or no inhibition in the activity of *S. faecalis* lipase. These results are comparable to the findings of Dirks *et al.* (1955), Patel *et al.* (1964), Somkuti *et al.* (1969) and Oterholm *et al.* (1970).

While studying the effect of EDTA on the activity of *Propionibacterium shermanii* lipase, Oterholm *et al.* (1970) suggested that the rate at which the above enzyme hydrolysed triglycerides may depend upon the interfacial area between emulsified substrate and soluble enzyme and the agents which reduce the interfacial area may thus act as inhibitors. The inhibitory action of a surface active agent like sodium lauryl sulphate on the activity of *S. faecalis* lipase may have to be explained in the above manner. *Mucor* lipase was also inhibited by sodium lauryl sulphate (Nagaoka and Yamada, 1973). Reduction in activity of *S. faecalis* lipase by potassium permanganate as reported in the present study is comparable to a similar observation by Nagaoka and Yamada (1973) on *Mucor* lipase.

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