1. Roy's liquid medium supports growth of *L. donovani* promastigotes. The mean generation time of the organism in this medium is 7.3 h and exponential phase of growth is maintained for more than 80 h. Rabbit blood hemolysate and beef heart infusion are two chemically highly complex ingredients of this medium.

2. There was no specificity for the source of blood used for hemolysate preparation. Guineapig blood served as good as the rabbit blood. Human blood was also similarly effective.

3. The protein fraction of the blood hemolysate was ineffective in supporting the growth of *L. donovani* promastigotes in the blood lysate deficient medium.

4. Almost normal growth of the protozoon was supported if the blood lysate deficient medium was supplemented with corresponding hemin fraction.
5. *L. donovani* growth stimulatory factors of beef heart infusion (BHI) was nondialysable and was soluble in conventional lipid solvents.

6. The growth promoting factors in BHI were polar lipids containing choline namely phosphatidyl choline and lysophosphatidyl choline. All other lipid fractions of BHI were unable to replace BHI in Roy's liquid medium.

7. The growth supporting effect of phosphatidyl choline and lysophosphatidyl choline was retained when chemically hydrolysed products of them were used. Out of different components of phosphatidyl choline, choline was identified as the *L. donovani* growth factor.

8. The modified Roy's medium, designated as GC I medium, as was formulated by replacement of blood lysate and BHI of Roy's medium with hemin and choline chloride respectively and by further fortification of the medium with micronutrients like folic acid and inorganic salts contained per liter: glucose, 10 g; peptone (Oxoid, L37), 10 g; NaCl, 6 g; Choline chloride, 5 mg; folic acid 1 mg, hemin, 1 mg;
Chapter 2

1. D-glucose, D-galactose, D-mannose, D-fructose, maltose, sucrose, mannitol and glycerol could support the growth of *L. donovani* promastigotes in the GC I medium but sorbitol, inulin, lactose or starch could not.

2. Among different sugars and sugar derivatives tested D-glucose was identified as the most efficient growth supporter of this protozoon.

3. Malt extract (Oxoid, L39) could support the growth of *L. donovani* promastigotes in the GC I medium better than glucose under identical conditions.

4. When malt extract was used extra additions of choline chloride, folic acid, and inorganic salts other than NaCl was unnecessary.

5. Promastigotes of *L. donovani* could not grow in a ba-
sal medium containing only malt extract, NaCl and hemin. Addition of inorganic nitrogen compounds in the basal medium also could not support any growth of the protozoon.

6. Among the complex nitrogen sources tested, Peptone (Oxoid, L37) was identified as the most efficient nitrogen source in supporting the growth of L. donovani in the basal medium.

7. NaCl of the medium could be replaced by KCl but not by NH₄Cl or any other inorganic salts.

8. The newly formulated complex growth medium for L. donovani promastigotes by modification of the GC I medium contained per liter: malt extract (Oxoid, L39), 5 g; NaCl, 4 g; Peptone (Oxoid, L37), 10 g; hemin, 600 mg and glass distilled water, 1 liter. For the solid medium preparation malt extract was used 10 g lit⁻¹ and bacteriological agar powder (Difco), 1.3 g l⁻¹ was added.

9. The newly formulated growth medium was designated as MEP medium and L. donovani promastigotes grow in this medium optimally at pH 7.5 and at 25°C.
Chapter 3

1. Replacement of peptone (Oxoid, L37) of GC I medium by casaminoacid (Difco) could not support the growth of the promastigotes of *L. donovani* in this medium. Fortification of this casaminoacid containing medium with L-tryptophan, different water soluble vitamins and adenosine supported the growth of this protozoon.

2. An external buffering system was essential to be added to the newly formulated protein and lipid free chemically semidefined medium, designated as GC II medium. Among the different buffering systems tested HEPES buffer was found to be the most suitable one.

Chapter 4.

1. The casaminoacid component of the GC II medium was replaced by a mixture of chemically pure aminoacids whose composition was identical to the composition of casaminoacid. Thus a new chemically defined medium for *L. donovani* promastigotes, designated as GC IIIa, was formulated.

2. D-glucose was the most suitable energy source for *L. donovani* promastigotes among the sugars and su-
gar derivatives tested in the chemically defined GC IIIa medium.

3. D-glucose, D-fructose, D-mannose and D-galactose were among the hexoses and D-ribose and D-xylose were among the pentoses which were utilised by the protozoon in the chemically defined GC IIIa medium.

4. Among the disaccharides tested, maitose and sucrose could support the growth of the promastigotes of *L. donovani* but poorly. Sugar alcohols, oligo and poly-saccharides tested were not utilised from the chemically defined medium.

5. Among the organic acids tested only succinic acid could support the growth of *L. donovani*.

6. L-aspartic acid, L-asparagine and L-glutamic acid were better growth supported than D-glucose in the chemically defined GC IIIa medium. Among all the carbon compounds tested, L-aspartic acid was found to be the most suitable energy molecule for the *L. donovani* promastigotes in the GC IIIa medium.

7. For its active growth the promastigote of *L. donov-
ani in GC IIIa medium requires nicotinamide, folic acid, thiamine hydrochloride, pyridoxine hydrochloride, calcium pantothenate, D-biotin, riboflavin and choline chloride. However, lipoic acid and m-inositol were not needed.

8. Thus the GC IIIa medium was modified to obtain a new chemically defined medium, designated as GC III medium, by replacing D-glucose of GC IIIa medium by equivalent amount of L-aspartic acid and omitting lipoic acid and m-inositol from the medium.

SECTION B

Chapter 1

1. The suspension of the promastigotes of L. donovani in PBS gradually lost their motility upon incubation at elevated temperature (37°C). Addition of readily utilisable energy source such as glucose to the incubation mixture decreased the rate of loss of motility.

2. The log phase cells took more time than the stationary phase cells to loss their motility.
3. Incubation of the promastigotes at a temperature above 37°C accelerated the process of motility loss. The motility rate of the cells was optimum at 25°C; decrease in the incubation temperature from 25°C to 15°C caused significant decreases in the rate of motility of the cells.

4. When exposed to 37°C the characteristic elongated shape of the promastigotes was lost and the cells assumed almost amastigote-like ovoid shape with shortening of the flagellum.

5. No such temperature induced morphological changes occurred when washed cells of *L. donovani* promastigotes were incubated in nutrient free isotonic PBS at 37°C. The cells lost their motility and were eventually killed at this situation.

6. For the effective morphological transformation of the promastigotes of *L. donovani* in vitro the presence of glucose and aminoacids were essentially required. Growth factors like vitamins, minerals and adenosine did not affect the rate of morphological transformation at 37°C.
7. For the resting cells of *L. donovani* the range of heat killing at 37°C was uniform throughout the period tested. For the growing cells this death rate was very slow initially but increased sharply during subsequent time of incubation depending upon the availability of readily utilisable energy source in the incubation mixture.

8. The promastigotes of *L. donovani* lost their motility and changed their morphology to the ovoid shape even at 25°C when were incubated under anaerobic conditions. The process of morphological transformation was accelerated if the temperature of incubation was elevated to 37°C during the anaerobic treatment of the cells.

9. The process of morphological transformation as is induced by elevated temperature in the growing cells of *L. donovani* promastigotes was retarded if the reaction mixture is aerated during the heat-shock.

10. The growing cells of *L. donovani* promastigotes were transformed morphologically to nonmotile ovoid bodies only at pH 5 or below when incubated at 25°C. However these nonmotile morphologically transformed
cells were mostly nonviable. the morphology of the promastigotes was not altered when the cells were allowed to grow at pH above 5.8 at 25°C.

11. The process of temperature induced transformation in the morphology and motility of the promastigotes of \textit{L. donovani} was accelerated when the pH of the medium was acidic as compared to that at higher pH. However the viability of the morphologically transformed cells decreased with decrease in the pH of the growth medium.

12. Inhibitors of energy metabolism of the cells catalysed the change in the morphology of the growing cells of \textit{L. donovani} promastigotes at 25°C. The resting cells were simply killed without any significant loss in morphology of the cells.

\textbf{Chapter 2}

1. Increase in incubation temperature of the resting cells of \textit{L. donovani} promastigotes resulted in increase in the leakage of metabolites having absorbance at 260 nm and 280 nm.

2. The rate of metabolite leakage by the resting cells
of *L. donovani* promastigotes were higher at 37°C than at 25°C.

3. The uptake of the essential metabolites like D-glucose, L-leucine and uracil showed saturation kinetics with respect to substrate concentration.

4. Kinetic studies showed that the rate of transport of these compounds decreased with increase in incubation temperature from 25°C to 37°C.

5. Both at 25°C and at 37°C the transport rates of these three metabolites were stimulated by the saturation of the reaction mixture with oxygen and were inhibited by deoxygenation.

6. The inhibitory effect of elevated temperature (37°C) on the transport processes was counteracted if the incubation mixture was aerated vigorously during incubation.

7. Addition of readily utilisable energy source such as glucose to the reaction mixture for the transport assays further stimulated the transport rates of L-leucine and uracil.
8. The transport of D-glucose, L-leucine and uracil was inhibited by the inhibitors of energy metabolism of the promastigotes of L. donovani.

Chapter 3

1. The rate of in vivo RNA synthesis in both the growing and the resting cells of L. donovani promastigotes was decreased significantly by the shift of medium temperature from 25°C to 37°C.

2. The rate of RNA synthesis in vivo was increased by the addition of glucose to the resting cells. RNA synthesis was also stimulated by increased oxygen supply to the reaction mixture in the resting cells as well as the growing cells of L. donovani promastigotes.

3. By the shift of medium temperature from 25°C to 37°C the rate of in vivo protein synthesis in L. donovani promastigotes was decreased significantly. However, introduction of excess oxygen or addition of glucose (in case of resting cells only) to the medium resulted in the stimulation of protein synthesis.

4. The rates of protein biosynthesis in vitro, cataly-
sed by the kinetoplast-mitochondrial fraction and the microsome-cytosol fraction of *L. donovani* promastigote cell extract were increased with increase in incubation temperature from 25°C to 37°C.

5. **In vitro** protein biosynthesis by the kinetoplast-mitochondrial fraction of *L. donovani* promastigotes was inhibited by the antibacterial antibiotics such as tetracycline, chloramphenicol, streptomycin but not by kanamycin or cycloheximide, a well-known eukaryotic protein synthesis inhibitor.

6. Protein synthesis in the microsome-cytosol fraction of *L. donovani* promastigotes was not inhibited by the antibacterial antibiotics tested but was inhibited by cycloheximide.

**SECTION C**

**Chapter 1**

1. About 13.5% of the cell dry weight of *L. donovani* promastigotes (Indian strain) was constituted by lipids. Neutral lipids and phospholipids constituted about 23% and 76% respectively of the total lipids. The ratio of phospholipids to neutral lipids was 3.3.
2. Sterols constituted about 1.4% of cell dry weight, about 10% of the total lipids and about 43.5% of the total neutral lipids of *L. donovani* promastigotes. The ratio of phospholipids to total sterol was 7.6.

3. Free sterol constituted major part of the sterols of *L. donovani* promastigotes. The ratio of free sterol to sterol esters was 2.0.

4. Ergosterol was identified as the main sterol in the promastigotes of *L. donovani*.

5. The nonsterol neutral lipids of *L. donovani* promastigotes was constituted by neutral plasmalogens, neutral glycerides and free fatty acids.

6. Among the diacylglycerols of *L. donovani* promastigotes the 1,3-derivative predominated over the 1,2-derivative.

7. Triacyl glycerol of *L. donovani* promastigotes constituted about 14.5% of the total neutral lipids.

8. Phosphatidyl choline, lysophosphatidyl choline, ph-
osphatidyl ethanolamine, phosphatidyl inositol, phosphatidic acid and diphosphatidyl glycerol are the phosphatides detected in the promastigotes of *L. donovani*. Among them the choline containing lipids constituted the major part.

9. Among the ethanolamine phospholipids a significant amount was constituted by ether lipids. Other phospholipids with detectable amount of ether lipids was the inositol phospholipids. Phosphatidyl choline and diphosphatidyl glycerol did not have ether lipids associated with them.

Chapter 2

1. In total lipid fraction of *L. donovani* promastigotes oleic acid was the most abundant fatty acid. Linoleic and linolenic acids were also present in high amount. Among the saturated fatty acids, palmitic acid was the most abundant. Other saturated fatty acids detected were lauric, myristic and stearic acids.

2. Fatty acid profile of the triacyl glycerol fraction were almost similar to that of the total lipid fraction.
3. Linolenic acid was the most abundant fatty acid in the diacyl glycerol fraction which also contained significant amount of linoleic and oleic acids. Among saturated fatty acids, stearic acids predominated over palmitic acid in this fraction.

4. In phosphatidyl ethanolamines of *L. donovani* promastigotes, linoleic acid was predominating over oleic acid; linolenic acid was present in relatively small amount.

5. In the phosphatidyl choline fraction, linoleic and linolenic acids predominated over oleic acid.

6. In phosphatidyl inositols, oleic acid and stearic acids were the main fatty acids.

**Chapter 3**

1. The spleen tissue of golden hamster is less lipid rich than *L. donovani* promastigote cells.

2. Phospholipid content is much higher in *L. donovani* cells than in the hamster spleen cells where as the latter is rich in the neutral lipid content than the former. The ratio of phospholipids to neutral lipids
is 0.94 in hamster spleen and is 3.27 in \textit{L. donovani} promastigotes.

3. Sterol content of hamster spleen tissue is very high in comparison to that of the promastigotes of \textit{L. donovani}. Phospholipid to total sterol ratio is 2.2 in hamster spleen tissue and 7.5 in the promastigotes of \textit{L. donovani}.

4. Main sterol compound present in hamster spleen tissue is cholesterol whereas the main sterol in the promastigotes of \textit{L. donovani} is ergosterol.

5. Free sterol to sterol ester ratio in hamster spleen tissue is 9.0 and that in the promastigotes of \textit{L. donovani} is 2.0.

6. Hamster spleen tissue contains higher amounts of triacyl glycerols than \textit{L. donovani} promastigotes. On the other hand \textit{L. donovani} cells contain higher amounts of neutral plasmalogens, diacyl glycerols and free fatty acids than hamster spleen tissue.

7. 1,3-diacyl glycerol is remarkably higher in \textit{L. donovani} cells. The ratio of 1,2-diacyl glycerol to 1,3-
-diacyl glycerols is 2.0 in hamster spleen tissue and is 0.3 in *L. donovani* promastigotes.

8. Relative concentration of phosphatidyl ethanolamine, phosphatidic acid and diphosphatidyl glycerol are higher in *L. donovani* cells in comparison to those in the hamster spleen tissue. Ratio of phosphatidyl choline to phosphatidyl ethanolamine is 0.25 in hamster spleen tissue and 0.7 in *L. donovani* promastigotes.

9. Hamster spleen tissue contains trace amounts of lysophosphatidyl choline whereas lysophosphatidyl choline contribute significantly to the phospholipids of *L. donovani* promastigotes.

Chapter 4

1. Lipid content of the promastigotes of *L. donovani* was increased significantly during the exposure of the cells to 37°C. There was no significant alteration in the phospholipid content (expressed as % cell dry weight) but the neutral lipid content of cells increased due to the exposure of the growing cells to elevated temperature.
2. Among the lipids, the relative amount of phospholipids (expressed as % total lipids) decreased and that of increased with increase in incubation temperature of the medium of the promastigotes of L. donovani resulting in a shift in the ratio of phospholipids to neutral lipids from 3.3 to 2.2.

3. There were significant increase in total sterol content with the increase in both free sterol and esterified sterol content of the promastigotes of L. donovani. During the exposure of the cells to 37°C the ratio of free sterol to sterol esters changed from 2.0 to 1.1.

4. Amount of all nonsterol neutral lipids detected in L. donovani promastigotes, but free fatty acids, increased due to increase in incubation temperature from 25°C to 37°C. Free fatty acid content of the cells remained unaffected.

5. The relative abundance of phosphatidyl choline decreased but those of phosphatidyl inositol and lyso-phosphatidyl choline increased in L. donovani cells when exposed to 37°C for 20 h. There were no or little change in phosphatidyl ethanolamine, phosphatidic acid and diphosphatidyl glycerol content of the cells due to increase in incubation temperature.
6. Relative amount of palmitic, stearic and oleic acid in the total lipid fraction increased but that of all other polyunsaturated fatty acids notably linolenic acid decreased during the shift to higher incubation temperature.

7. Total lipid content of the promastigotes of *L. donovani* decreased when the culture was aerated vigorously but increased when the cells were incubated under anaerobic conditions. The ratio of total phospholipid to total neutral lipids did not alter due to aeration of the culture but decreased significantly in the anaerobic culture.

8. Total sterol content of *L. donovani* promastigotes was increased due to vigorous aeration as well as due to anaerobiosis of the culture. The ratio of free sterol to sterol ester increased in the shake culture but this ratio remained unaltered in anaerobically growing cells. Phospholipid to total sterol ratio decreased both due to vigorous shaking of the culture and anaerobiosis; the decrease was more with anaerobically grown cells.

9. All the nonsterol neutral lipid classes including the neutral plasmalogens, diacyl and triacyl glyce-
rols and free fatty acids in the cells were decreased due to vigorous shaking of the culture—the decrease in the free fatty acid content was the most significant.

10. The content of nonsterol neutral lipid compounds except free fatty acids were increased when the promastigotes of *L. donovani* were allowed to grow under anaerobic conditions at 25°C. The increase in triacyl glycerol content and decrease in free fatty acid content of the cells were significant.

11. When the culture of *L. donovani* promastigotes was aerated vigorously, the content of phosphatidyl inositol, lysophosphatidyl choline and diphosphatidyl glycerol decreased significantly, the content of phosphatidyl choline rose sharply and the content of phosphatidyl ethanolamine and phosphatidic acid remained unaltered.

12. Anaerobiosis caused significant decrease in the amount of choline containing lipids in the promastigotes of *L. donovani*; the content of phosphatidyl ethanolamine, phosphatidic acid and diphosphatidyl glycerol were increased and phosphatidyl ethanolamine...
inositol content remained unaltered during anaerobiosis of the culture.

13. No significant change in the gross lipid composition of the promastigotes of *L. donovani* did occur due to the change in the hydrogen ion concentration of the growth medium.

14. The lipid content of the promastigotes of *L. donovani* increased with increase in the lipid content of the growth medium. The contents of phospholipids and neutral lipids also was higher in cells grown in lipid rich complex medium than that of the cells grown in lipid free chemically defined medium.

15. Total lipid content of the promastigotes of *L. donovani* increased with increase in culture age. This increase in lipid content was mainly due to increase in neutral lipid content of the cells; phospholipid content of the cells decreased with increase in culture age.

**Chapter 5**

1. Lipid composition of the nuclear, mitochondrial and plasmamembrane fractions of *L. donovani* promastigo-
tes were analysed. The mitochondrial fraction was more lipid rich than the nuclea and the plasmamemb- 
rane fraction. Neutral lipids were predominating component in all subcellular fractions tested.

2. Ratio of phospholipid to total sterols in the nucl-
ar, plasmamembrane and the mitochondrial fraction were 2.96, 0.66 and 1.16 respectively.

3. Triacyl glycerol content of the nuclear and the mi-
tochondrial fraction was similar and was more than that of the plasmamembrane fraction.

4. In the plasmamembrane fraction phosphatidyl ethanol-
amine was predominating over all phospholipids; ph-
osphatidyl choline and phosphatidyl inositol were also present in significant amounts.

5. In the nuclear and the mitochondrial fractions cho-
line containing lipids were dominating over other phospholipid classes.

Chapter 6

1. Olive oil substrate could not be hydrolysed by the cell free extract of L. donovani promastigotes.
2. Different phospholipase activities were detected in the cell free lysate of *L. donovani* promastigotes which included phospholipase A, B, C, and D. Activities of all these phospholipases were stimulated by detergents and were inhibited by increase in incubation temperature from 25°C to 37°C.

3. Phosphatidate phosphatase and choline kinase activities were also detected in the cell free lysate of *L. donovani* promastigotes. Activity of phosphatidate phosphatase decreased with increase in incubation temperature from 25°C to 37°C whereas reverse was true for choline kinase.

Chapter 7

1. Activities of acid and alkaline phosphatases and inorganic pyrophosphatase were detected in the cell free lysate of *L. donovani* promastigotes.

2. The activities of all these phosphohydrolases were increased sharply with rise in incubation temperature from 25°C to 37°C.