Introduction
CHAPTER I

INTRODUCTION :

Fats and Oils play a very significant role in nutrition and physiology of animals. Unsaturated fats have lower melting points and remain in liquid form at room temperature. The iodine number is a measure of the degree of unsaturated of fats and is defined as the grams of iodine absorbed by 100 gms fat. These unsaturated fats are important constituents of the dietary requirement because they have higher energy value and are the source of chemicals that are essentially for growth and the repair of worn out cells and tissues. Burr and Burr 1929, Deuel et al 1947, Deuel et al 1955 a. Added fat may increase the palatability of foods by absorbing and retaining flavour. As they tend to be digested slowly, they produce a feeling of satiety. (Burr G.O. et al (1930), Swift R.W 1952, Deuel et al (1955 a), Peiffer J.J. et al 1959, Holmen RT (1964).

A nutrition is considered essential if its deficiency results in recognizable clinical symptoms that are relieved by adding it to the diet. The goals of nutritional science are to define for
each individual, the complete set of nutrients required in the diet, the optimal amount of each nutrient and the combination of foods that best meets these requirements. Determination of how these requirements vary throughout normal life cycle and to understand nutritional factors are affected by injury, illness and treatment have not yet been entirely achieved.

Most studies on nutrition have emphasized the biological and physio pathological aspects of the individual. According to Barki V.H et al 1947, Aaes Jorgensen E et al 1954, Berg B.N. 1960, Holmen R.T. 1960, Ross M.H. et al 1975, Gopalan C et al (1980) a minimum amount of lipid is essential in the diet to provide an adequate supply of certain polyunsaturated fatty acids (PUFA) and of fat soluble vitamins that can not be synthesized in adequate amounts for optimal body function. Growth reproduction, behaviour patterns, nutrient storage, enzyme activity and gross histological appearances of tissues and their contents of nucleic acids and proteins are the major criteria used to assess nutritional adequacy of diets. Ideal performance is not known in all cases. Greatest consumption of nutrients and rapid growth often do not correlate with the longest life span and freedom from diseases.
Many diets for rats are composed of natural ingredients and are conveniently obtained from commercial sources (Ross, M. H. and G. Bras 1975). The data on which the requirements are based were reported from many different laboratories that operate under varying conditions of diet mixing and storage, rat strain, handling etc. These recommendations may be assumed to be adequate for rats in different laboratory conditions. However experimental procedures and environmental conditions may alter the requirements for one or more nutrients. Dietary fat has two functions in the body of the animal -

I. It acts as a solvent for the absorption of fat soluble vitamins and

II. It provides the essential fatty acid i.e. - linoleic acid. Fat tends to reduce the dustiness of foods which may increase the acceptability of high roughage diets.

Vegetable oils are almost 100% fat and are rich in unsaturated fatty acids. Among the unsaturated fats the safflower oil rich in linoleic acid is an essential fatty acid (EFA). Linoleic acid, linolenic and arachidonic acids are the only fatty acids known to be essential for the complete nutrition
of many animals and thus must be supplied in the diet and are known as nutritionally essential fatty acids. (Holmen R T 1960).

Linoleic acid is widely distributed in the lipid portion of both plants and animal foods. Vegetable seed oils are especially rich sources of linoleic acid. From the literature it appears that an optimum amount of EFA in the diet of rat is perhaps beneficial as regards the general functions and proper growth of the animal. (Barki et al 1950). But how much of the essential unsaturated fat is needed in the diet has not been determined. Moreover, the symptoms of EFA deficiency have been prevented by feeding diet containing 1 to 2% of the metabolizable energy in the form of linoleic acid (Greenberg et al 1970).

Linoleic acid is the most important of the polyethenoid acids found in vegetable oils. The commercial saffola oil in the safflower seed oil rich in linoleic acid (70%) (Betschart A A et al, Weiss E A 1983). The generally accepted formula for linoleic acid is of 9,12-octodecadienoic acid. The presence of unsaturated bonds enables linoleic acid to take up oxygen from the air. The acid which has more than one double bond is of great importance in animal nutrition. They include the so called "essential"
fatty acids which are required by the animal since they cannot be synthesized from other fatty acids or carbohydrates. (Deuel et al 1947).

The requirement of EFA is usually expressed in terms of linoleic acid with the greatest biological activity. The early work of Burr and Burr (1929) demonstrated the essentiality of dietary fat. Polyunsaturated fatty acids mainly linoleic acid were shown by Burr and Burr (1930) to combat the adverse effect of fat free diets. Three polyunsaturated fatty acids usually referred to as EFA are linoleic acid (18 : 3n - 3) and arachidonic acid (20 : 4n-6). Arachidonic acid has the highest bipotency for growth (Holman 1968). Arachidonic acid itself is not essential; it can be derived from linoleic acid (sprecher 1972), γ-linoleic (18 : 3n6) and decosapentaenioic (22 : 5n6) acids (Holman 1970). Holman (1968) extensively reviewed the EFA literature and found that a number of factors affect development of deficiency under laboratory conditions. In recent years the controversy over the relationship of various dietary factors and the deficiency syndrome has promoted research interest on this line Trugnan G. G. et al (1985), Akhtar et al (1987) Anderson et al (1985), Harris M. I. et al (1987).
Liver has a great role to play in nutrition as a whole. It has also a key role in the metabolism of fat. Therefore liver of rat has been selected as one of the target organs for the study of unsalurated fat in the present investigation. The liver of rat is a structure of complex nature performing a number of functions essential to the physiology of the body and forms a major site for the transportations of fatty acids. Both in adipose tissue and also in liver, fats are always in a state of continuous flux as they move from the fat deposits to the liver and vice versa. Some of the body fat is oxidized to yield energy and whenever the fat consumed by way of diet is in excess of the fat oxidised, it is stored in the body. Pearson et al (1949) Peifer J. J. et al (1959) Sprecher H. W. (1972) Gopalan et al (1980).

The concept of a central and unique role for the liver in lipid metabolism is still an important subject. Liver plays an important role in facilitating the digestion and absorption of lipids by the production of bile, which contains cholesterol and bile salts synthesized within the liver. The liver has active enzyme systems for synthesizing triacyl glycerols, phospholipids, cholesterol and plasmalipoproteins and for converting fatty acids to ketone bodies through a process of ketogenesis. For a
variety of reasons lipid can accumulate in the liver mainly as triacylglycerol. Extensive accumulation is regarded as a pathologic condition. When accumulation of lipid in the liver becomes chronic, fibrotic changes occur in the cells that progress to cirrhosis and impaired liver function (Masoro 1977). It is well known that a well functioning liver with high percentage of glycogen is more resistant to various dietary factors. The metabolism of fat in living tissue is carried on by a diverse and intricate mosaic of enzymic catalysis. Under normal condition each tissue maintains a steady and consistent enzymic pattern. The significance of enzymic regulation must be understood in terms of the organism's necessity to maintain homeostatic balance among varying internal and external conditions. One of the most outstanding characteristics of the organism is its ability to adopt rapidly and success fully to continuously changing physiological and pathological circumstances. Adaptation at cellular levels increases or decreases the rates of certain metabolic processes in the organ.

The kidney plays an important role in fat utilization and is vitally important for homeostasis. A number of workers reported a significant decrease in kidney phospholipids and liver phospholipids, Aoyama, Yoritika et al (1979), Miur,
Moriyuki (1985), Pearson et al (1991). When animals were subjected to a choline deficient diet. In the case of rat the normal level of free choline varies between 1.3 and 1.9 Hg/ml. However when this species was maintained for four months on a choline deficient diet the plasma level fell between 0.6 and 1.1 Hg/ml (microgram/ml). Under normal conditions when choline is injected, it is rapidly removed from the blood. Choline is a growth factor for rats and certain other laboratory animals. Choline is considered under the B vitamins which is essential for normal nutrition of many animal species. But deficiency symptoms cannot be produced on diets devoid only of Choline when other molecules for the synthesis of Choline trimethylethanol ammonium hydroxide are present (West and Todd 1956). Ethylol trimethylammonium, \( \text{OH CH CH}_2 \text{N(CH}_3\text{)}_2 \text{OH} \), an amino base present in the blood; cerebrospinal fluid and urine, may be extracted from many of the body tissues including skeletal and cardiac muscle. It has a lipotropic action and disperses fat from the liver or prevents its accumulation in excess.

Although perirenal depots represent an important site for the storage of neutral fat, the proportion of this component within the kidney is minimal (Deuel H. J. 1955). More than 85% of the
lipid in the kidneys of the older rats was composed of essential lipids. The distribution of essential lipids in the kidneys of the mature rats is as follows:

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipids</td>
<td>15.19%</td>
</tr>
<tr>
<td>Cerebrosides</td>
<td>1.30%</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>1.00%</td>
</tr>
<tr>
<td>Cholesterol ester</td>
<td>0.94%</td>
</tr>
<tr>
<td>Phospholipids were composed of</td>
<td></td>
</tr>
<tr>
<td>Cephalin</td>
<td>7.40%</td>
</tr>
<tr>
<td>Lecithin</td>
<td>5.96%</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>1.83%</td>
</tr>
</tbody>
</table>

That cholesterol is synthesized in the kidney is known from the work demonstrated by Srere et al (1950) in rat kidney. A slight increase in the proportion of total lipids in the kidney occurs with increasing age. One of the most important factors concerned with the nature of the kidney lipids is diet. The kidneys are almost as active as the liver in the incorporation of ingested fatty acids into the phospholipids. Perlman et al (1937) found that the maximum level of labeled phospholipid was reached in the kidney about 15 hours after it was fed, while it required only 10 hrs to reach the peak in the case of liver. The phospholipid was retained longer in the kidney than in the liver. Similar results were reported by Artom et al (1937). A kidney injury some
what similar to that resulting from Choline deficiency has been reported in rats which have received a diet devoid of essential acids over a prolonged period. On a diet low in protein and Choline, rats develop a type of liver cirrhosis which is completely prevented by including Choline in the diet. (Ross et al 1995, Carroll K. K. 1980). In this case also the injury appears to be related to the inability of the rat to synthesize enough phospholipids to serve as the structural portion of the kidney when the essential fatty acids are absent from the diet. Although large amounts of fat associated with the kidney consists of perirenal fat located outside of the organ, definite concentrations occur within the kidney itself. The concentration of fat increased with age and its location and the size of the intracellular fat globules varied widely with increasing age. Ross M. H. et al (1975) Sprecher H. W. 1972. Aaes - Jorgensen E et al (1954).

A severe choline deficiency in young rats brings about a necrosis of the kidney and a fatty degeneration of the liver. these changes are reversible on the administration of choline or other lipotrophic agents (West and Todd 1956).
The composition of blood is considerably influenced by the ingestion of food. The blood lipids are in a continuous stage of change and these are added to the blood by absorptions from the intestine, by synthesis, particularly by the liver and by mobilization from the fat depots. Since the lipid compositions of plasma and cells are widely different and plasma lipids more accurately reflect the state of lipid metabolism, it is customary to use only the plasma for analysis. Many factors influence the quantity and distribution of blood lipid (West a Todd 1956).

In recent years the controversy over the relationship of various dietary factors and the incidence of atherosclerosis and coronary disease has promoted research interest on the effect of various dietary factors on the circulating lipids. Numerous control experiments on humans have been conducted to determine the effect of dietary fat on the concentration of lipids in the blood serum. Since 1959 attention has been given to several items that were not considered in the earlier analysis. These include dietary cholesterol and certain individual fatty acids (Seelys 1988, singh R. B. 1993, Hirot sugu U. 1982). Although the mechanism involved in the body remains obscure, it is generally agreed that the response of serum cholesterol to changes in the

The present investigation with dietary effect of safflower oil on male albine rat is also focussed on the changes in the biochemical analysis in terms of blood protein, blood glucose, serum alkaline phosphatase activities and plasma cholesterol levels. Since the levels of the several lipids in the blood mirror the conditions in the tissues, any abnormality which results in variations in the blood picture is usually to be ascribed to an alteration of the metabolic breakdown in the tissue themselves.

The plasma contains several types of proteins which remain combined together forming a single protein complex. Plasma protein can be synthesized from amino acids. The synthesis of serum albumin, prothrombin, and fibrinogen occurs solely in the liver parenchyma. The plasma proteins may be decreased as the result of dietary deficiencies, hemorrhage, and in nephrosis due to the large reduction in albumin in liver diseases which interfere with plasma protein formation. Many diseases are
characterised by alterations in plasma albumin, globulin and fibrinogen values with little or no abnormality in the quantity of total plasma proteins. The chief function of plasma proteins are -

1. To provide resistance to blood flow in the vascular system.
2. To serve as a source of nutrition for the tissues of the body.
3. To regulate the distribution of fluid between the blood and tissues.
4. Plasma proteins provide for the control of hemorrhage through mechanisms for blood coagulation.
5. They contribute to the solution and transport of lipids, for soluble vitamins, bile salts, hormones and various drugs in the blood through the formation of complexes.
6. Plasma proteins provide antibodies for defense against infection.

Therefore, in the present investigation it is proposed to estimate the plasma protein levels of blood which occur due to unsaturated fat supplemented diet on male albino rat.

In the present experiment with unsaturated fat diet on male albino rat, the biochemical changes produced in the blood glucose level was observed. Insulin effects the rate of glucose transport. Glucose can be used immediately for release of energy to the cells or it can be stored in the form of glycogen. When glucose is not immediately required for energy, the extra glucose
that continually enters the cells either is stored as glycogen or converted into fat. When the body's storages of carbohydrates decrease below normal, moderate quantities of glucose can be formed from aminoacids and from the glycerol protein of fat. Rise of blood glucose means the rise of glycogen storage in liver and in other muscles resulting in conversion to fat for storage in adipose tissue. Glycogen in the storage polysaccharide which serves as energy reservoir in animal and plants. Liver glycogen plays a very important role in regulating the blood sugar level and serves as glucose reserve. When blood sugar level is plentiful it converts to glycogen and when blood sugar falls, glycogen can convert to blood glucose. The breakdown of liver glycogen is promoted by the hormone epinephrine. The tissues utilise glucose for glycogen formation, fat formation and ultimately energy production through oxidation. Fain J. N. (1984) Guyton J. R. et al (1978), Kraus-Friedman N (1984), Wilsen et al (1986).

The blood glucose level varies continually with in limits and is determined by the balance between processes adding glucose to the blood gluconeogenesis and processes taking glucose from the blood. Formation of tissue glycogen production of fat and oxidation). If sugar utilization of the body
decreases, the blood sugar level is elevated such as in diabetes and when sugar utilization of the body increases, the blood sugar level is reduced as in hyperinsulinism which may indicate the various diseases of liver Hems D. A. (1978), Thompson A. B. (1987), Reaven G. M. et al (1986).

In the experiment, the effect of unsaturated fat providing the same level of linoleic acid on plasma cholesterol concentration was also studied. The data on which the requirements are based were reported from many different laboratories under varying conditions of diet mixing and storage, rat strain, handling etc. the effect of orally administrated unsaturated fat (safflower oil) on plasma cholesterol was studied in sixty healthy male albino rat of same age group. The purpose of present study is to observe the effect of commercial safflower oil on the plasma cholesterol level of rat fed with standard laboratory diet and to compare it with those fed with unsaturated fat free diet.

The name 'cholesterol' derived from the greek words meaning "Solid bile" is an essential constituent of all cells and body fluids. Windaus (1932) proposed a structure for cholesterol which is now generally accepted by all.

Chemical formula is \( \text{C}_{27}\text{H}_{45}\text{OH} \)
Cholesterol serves as the precursor of cholic acid of bile. In rat, a diet low in choline causes a great accumulation of rat. Choline seems to be a lipotropic factor. Cholesterol and its esters in the blood are essential to the body as they help in the repairing of the membranes, production of hormones and other purposes. Blood cholesterol content rises upto 700 mg under conditions like diabetes mellitus. Chobanian A.V. et al (1962), Holman R.R. (1980), Harris M.I. (1987). It has long been recognized that the fatty acids combined with cholesterol in the plasma are more unsaturated than are those in neutral fat or in phospholipids. Metabolism of cholesterol and that of the EFA are closely interrelated. It was first demonstrated by Alfin - Slater (1954) and her collaborators that the presence of EFA is required to aid in the regulation of the cholesterol in the blood and tissues. It is not known whether there is an impairment of cholesterol transport from the liver - when the linoleate is deficient, or whether the EFA help in the catabolism of cholesterol. Walker W.J. et al (1953) have reported that the increase in liver cholesterol was greater after cholesterol was fed to rats on an EFA free diet than on a normal diet. Cholesterol is absent in vegetable originated oil.
Alkaline phosphatase (Orthophosphoric monoester phosphohydrolase E.C. 3.13.1) is an enzyme which is determined according to its activity by standardised technique. The units of activity may be expressed per gram of dry enzyme preparations or per milligram of nitrogen contained. The phosphatase activity of blood serum is often measured as KA unit. The enzymes of diseased or damaged tissue may leak out into the blood. So the activity of an enzyme present in high concentration in the affected tissue, may rise significantly in the serum. Change in serum enzyme activities help in the diagnosis of diseases depending on -

(1) Higher concentration in the affected tissues or organs.
(2) The severity of damage of the tissues.
(3) Stage of maximum permeability and its durations.

For measuring an enzyme activity a measured amount of serum is mixed with co-factors, buffers and specific concentrations of its substrate and is incubated for a specific period and optical density of the product is estimated. Alkaline phosphatase hydrolyses organic phosphate esters to liberate inorganic phosphate at the serum PH of 7.4 although their optimum PH lies around 9. The total activity of isoenzymes in the normal serum amounts to 3 - 13 KA unit per 100 ml. Each KA unit indicates the
liberation of 1 mg pi per 100 ml serum on its incubation with βglycerophosphate for 1 hour at 37°C and PH 8.6.

Considering the above aspects it is proposed to study the effect of unsaturated fat supplement and deficient diet in a group of male albino rats and the results were compared with the control group of animal. Keeping in mind the importance of unsaturated fat, the present piece of work was undertaken with the following objectives -

(1) To study the effect of unsaturated fat supplement and deficient diet on morphology considering the growth in body weight.
(2) To study the effect of unsaturated fat supplement and deficient diet on histopathology in the liver and kidney tissue.
(3) To study the effect of unsaturated fat supplement and deficient diet on histochemistry in relation to DNA change, glycogen storage and alkaline phosphatase activity in the liver and kidney tissue.
(4) To study the effect of unsaturated fat supplement and deficient diet on the plasma glucose level.
(5) To study the effect of unsaturated fat supplement and deficient diet on the plasma protein level.
(6) To study the effect of unsaturated fat supplement
and deficient diet on the plasma cholesterol level. 
(7) To study the effect of unsaturated fat supplement 
and deficient diet on serum alkaline phosphatase 
activity.

The effect of unsaturated fat 
deficient and supplement diet on the morphological 
changes, histopathological, histochemical, and 
biochemical changes has been described in the chapters 
IV 'Experimental findings' and chapter IV A, IV B, IV 
C, IV D. In the general discussions attempt has been 
made to correlate the different findings of the 
present investigation and probable explanation has 
been put forwarded in Chapter V.

The findings of the present 
investigation will not only be useful in throwing some 
light on the problems of probable harmful effects of 
unsaturated fat deficient diet but also on the 
probable biochemical effects of unsaturated fat 
supplement diet on liver and kidney which are the 
metabolic houses of the body.