General Discussion
CHAPTER V
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The present study has provided some information about the supplementation of some amount of unsaturated fat in the diet of rat. As unsaturated fat has a source of essential fatty acids, the inclusion of this may help to combat the adverse effects of unsaturated fat deficient diet. According to Jorgensen et al 1954, Peifer and Holman 1959 diet containing more than 5 percent saturated fat may support a maximum performance. The early work of Burr and Burr (1929) also demonstrated the essentiality of dietary fat in the form of polyunsaturated fatty acids mainly linoleic acid. After a life time study in the field Deuel 1957 was convinced that some fat in addition to EFA is desirable for growth which was also supported by Pearson and Pauzer 1949, Greenberg et al 1950, Deuel 1957, Mohrhauler and holman 1967.

In the present investigation male albino rats were fed a supplement of safflower oil (0.5 ml/100 gm weight/day) for a period of one year and observations were made by comparing the effects with deficient group of rats that were fed with unsaturated fat free diet and control group with control diet (Table 1).
The results show (Table 3) that the mean growth rate for supplement groups (SI, SII, SIII, SIV) were higher than that of deficient groups. The loss of hair in the face and trunk started from early part of the experiment and became more prominent in 6 months (DII) and 9 months (DIII) in deficient groups. Death of two animals of deficient group one after 6 months and another after 9 months were also observed. At the same time, rats fed with supplemented diet were found to have a good health. The average weight of the animal in the supplement group evaluated in the laboratory condition was greater than that of deficient and control group of animals and is found to be highly significant (p < 0.001) as shown in table no. 4. The difference in the body weight of deficient and control group was found to be not significant. The both factors such as behaviour of the animals (activity) age of rats, component of deficient diet (Table 1) environmental condition of the locality, temperature (22°C - 36°C) and humidity (70% - 88%) of the laboratory, may help the animal to reduce the appearance of other symptoms as found by many investigators Burr and Burr 1929, Barki et al 1947, Deuel 1955, Berg B.N. 1960, Lowry R.R. et al 1966, Mohrhauer H. et al 1967, Sprecher H. W. 1972, Ross M. H. et al 1975.
The effect of unsaturated fat supplement diet on morphological changes also attracted the attention of a number of investigators in recent years, specially the fact that the lack of poly unsaturated fatty acids (PUFA) in animals and man showed down-growth and induced skin diseases. (Alimova et al 1975, Ramwell 1981).

Function of the different organs of the body are connected with its structural and physiological integrity. Any alteration in the dietary composition was also found to alter the structural and physiological integrity of the metabolic organs of the animals, mainly liver and kidney as they have the key role in the metabolism of fat. A careful anatomical study has revealed some of the changes in the structural integrity of the tissues in the deficient groups plate 4, 5, 6, 7 in liver and plate 13, 14, 15, 16 in kidney. Efforts were made to study the nature of fat accumulation in the cells by preparing frozen sections of the liver but due to temporary preparation of the slides the study could not give much reliable informations about the fatty degeneration of the cells and metabolic functional activity of the tissue. Electron microscopy is definitely one of the best modern tools for the study such histopathological activities of the cell and also
to recognize the subcellular components and their structural irregularities of the tissue. But due to lack of this facility, the study could not be carried out in this line.

Microscopic preparation and examination of the liver tissue from supplement group exhibited normal architecture of hepatic cells with clear central vein and portal canal area, binucleate hepatic cells with a few mitotic figures as shown in the plate 8, 9, 10, 11 which are more or less comparable to the control structure (plate 3) kidney tissue also shows the retentions of somewhat beautiful regular architecture as in plate 17, 18, 19, 20. Preparations of liver and kidney tissue of deficient groups show - plate 4, 5, 6, 7 and plate 13, 14, 15, 16 some irregularities in the arrangement of hepatocytes with disturbed portal canal area and irregularities in the glomerular structure with changes in capsular spaces, lightly stained degenerated cellular tuft, reduction of lumen of convoluted cells due to swelling of epithelial cells - plate 13, 14, 15, 16. The sign of unsaturated fat deficiency in the rat as found by many investigators in case of liver and kidney tissue plateaus after about 12 to 24 weeks by same chronic deficiency (Aaes Jorgen Sen et al 1954, Holman 1968, Sprecher H. W.
1972, Ross M. H. et al 1975). The findings in the present investigation also suggest that a supplementation of safflower oil of 0.5 ml/100 gm body weight/day is sufficient enough to restore the normal histological structure of the metabolic organs like liver and kidney in the experimental rats.

It was found in the literature that in same animals unsaturated fats accelerate tumor growth (carroll 1980, Hopkins et al 1981) with high rate of DNA synthesis. But in the present investigation with the supplement of only 0.5 ml/100 gm body weight/rat/day perhaps helps to overcome the deficiency syndrome. Also environmental circumstances may be a major factor in suppressing the occurrence of a high rate of DNA synthesis as shown in the plate 25, 26, 27, 28. In the present investigation with deficient diet some islands showing high rate of DNA synthesis in 6 months and 9 months as shown in plate 22, 23 and less in 12 months (plate 24) suggest that unsaturated fat deficient diet is not a major factor in development of liver lesions. Food supplies along with environmental circumstances may suppress the initial damage of the liver tissue (plate 22 and 23) and lead to repair in the later period (plate 24) in the present experiment.
The glycogen synthesis of liver varies under the influence of physiological pathological, nutritional and environmental condition, its accumulation depends on the rate of anabolic and catabolic reactions. Any disturbance in carbohydrate metabolism lead to accumulation or depletion of glycogen in the liver tissue. Light intensity of the PAS reaction for glycogen in liver indicates that metabolic system of glycogen storage and its mobilization in adult male rat may be influenced by unsaturated fat supplement diet. Some investigators also suggested (Holman 1968, Greenbery 1970) that 1 or 2% linoleic acid as metabolizable energy is effective for prevention of deficiency syndromes of unsaturated fat in most species. Less intensity of PAS reaction indicates less glycogen content as found in the supplement group. The accumulations of glycogen along with fat in the liver may be due to deficiency of required amounts of choline and EFA substances and thus it may be prevented by the supplement of unsaturated fat. The role of autonomic nervous system as described by authors, Shimazu and Fukuda (1965) may also affect the activities of liver enzymes which are concerned with carbohydrate metabolism in the regulations of glycogen accumulation in the tissue. In deficient liver sections particularly in the 6 months group high intensity of PAS reaction indicates
the rise of glycogen storage in the tissue which means the rise of blood glucose level also. Liver cells are congested with lipids and become longer, nucleus moves to the periphery and glycogen storage fluctuates towards decrease in 9 months and 12 months deficient groups which supports the findings of Colwell 1951, Panos and Finersty 1956 which might be seen in fatty liver due to the absence of lipotropic effect. Many authors explained the effect of cold environment in decreasing glycogen distribution, but conflicting results were put forward by them as to the irregular distribution of glycogen in the tissue in cold environment. (Kaufman et al 1958, Felts and Musor 1959, Depocus 1962). Fasting of 12 hour before sample collection enhances toxicity which probably leads to depletion of glycogen distribution in the present experimental groups which is also supported by Campbell et al 1971. According to Wiener et al 1968 and Badylak and Vanvleet 1982 powerful hormonal influences also control cell carbohydrate metabolism and usually underlie glycogen overload.

Increased alkaline phosphates activity in deficient groups as observed in the present investigation and less activity in the supplement groups suggest that the regulation of enzyme degradation might be related to the unsaturated
fat at definite concentration. Supplementation of unsaturated fat at the level of 0.5 ml/100 gm body weight/day may not be sufficient to reduce the activity of this enzyme to the normal level. The increased activity of this enzyme in supplement groups above control supports the work of Koshland D. E. et al 1968, Mildvan A. S. 1974, Wilfred 1984, Misumi et al 1986 that plant based vegetable products may be responsible for the increased activity of this enzyme. According to Pekarthy et al 1972 choline derivatives may raise the activity of this enzyme and also increase the enzyme concentration in the tissues. According to Weber 1963, Mildvan A. S. 1974, hormones, dietary components, age, induction, repression and environmental factors may also change the enzyme concentration in any mammalian tissue. Therefore the mild increase of enzyme activity in supplement group and its more increase in deficient groups may be associated with all these factors as well as histopathological organization of the tissue.

The biochemical study revealed the abnormalities in the process of metabolism concentration of vitamins and the activities of the enzymes in the blood. Blood regulates the metabolism of lipids and the transfer of its product from the tissue to another, for storage and to supply the
material for oxidation which is necessary to maintain the good health of the animals. There is a gradual elevation of plasma glucose level in deficient group upto 6 months and then the value declined towards the end of the experimental period. This may be due to the increased rate of absorption from intestine and increase in insulin level, glycogenesis and utilization of glucose in the liver. Gradual lowering of glucose level towards the end of the experiment may be due to increased oxidation of glucose and enhanced glycogenesis.

The clinical condition of elevation and declination may be regarded as a disproportion between requirements of the body and the secretion of hormones and enzymes as also explained by Fain J. N. 1984. According to Deuel et al 1947, Bloch K. et al 1977 the enhancement of plasma glucose level may be due to metabolic adaptation to unsaturated fat deficient diet which is also supported by the work of Cohen P. 1976, Hers H. G. 1976. According to Holman R. T. 1964 a deficiency of unsaturated fat appears to produce fatty liver in animals which is also responsible for abnormal carbohydrate metabolism and may be responsible for elevation of plasma glucose level. In the present investigation bangle gram being the only diet source and it is devoid of lipotropic
factors it may be responsible for initial elevation up to 6 months and as described by Akhtar et al. 1987 that legumes (traditional Pakistani meal) may help in the reduction of glucose level as seen towards the end of the experimental periods. This may be due to slight pancreatic insufficiency which would result in a raised blood sugar level to give a typical picture of uncontrolled diabetes which is also supported by the findings of Randle et al. 1967, Guyton J. R. et al. 1978, Holman R. R., Turner P. C. 1980, Fain J. N. 1984, Kraus Friedmen N. 1985, Harris M. I. et al. 1987, Reaven G. M. 1988. Slight elevation also observed in the supplemented groups and found more or less constant throughout the experimental period (122 mg/dl to 148 mg/dl) indicates that the rate of intake and output of glucose from extrahepatic tissue and liver shows a steady level. Though it is very difficult to pinpoint the exact mechanism involved for the changes of plasma glucose level in both deficient and supplemented groups above control value (100 mg/dl to 110 mg/dl) from the observations throughout the experiment in the present investigation it is clear that there might be some relation between carbohydrate metabolism and unsaturated fat in presence of bangle gram as the only source of diet.

Plasma cholesterol value increased
significantly in supplemented group then deficient and control value in the present observation during the experimental period. The elevation of plasma cholesterol in rats fed with unsaturated fat supplement diet was also observed by Keys and associates 1951, Holman et al 1959 in monkeys by Joagannathan S.N. 1962 and in men by Klein 1958. However the composition of basal diet seems to exert a decisive influence on the way in which dietary fat changes blood cholesterol level in rats. The result of various workers have given quite variable answers to the question as to whether or not the administration of fat causes a concomitant rise into blood cholesterol level. However Bloor W. R. 1915 in his earlier studies was unable to demonstrate any change in blood cholesterol following the feeding of fat. Most of the earlier data on human subjects failed to produce convincing evidence that hypercholesterolemia is a necessary concomitant of a fat meal. Hiller et al 1924, also noted only irregular increase in blood cholesterol after fat feeding and no uniformity in the cholesterol and fatty acid ratio was obtained. Turner and Stainer 1939, reported that the blood cholesterol in man is remarkably independent of food in take. On the otherhand Wendt H. 1932 found definite evidence that hypercholesterolemia followed the ingestion of oilve
oil, however the increase in cholesterol took place only in the plasma. Several reports indicate that a higher blood cholesterol may occur when the fat intake is high. But in the present experiment the composition of diet and safflower oil (Supplement oil) seems to exert an influence on the way by which dietary fat changes blood cholesterol level in rats. Hegsted 1957, Quackenbush F. W. 1960, Trugnan G. G. et al 1986, Huang Y. S. et al 1987 studied the effect of purified linoleic ester on rat and found that it is an effective agent in lowering cholesterol level in rats in hypercholesterolmic condition. In the present study, the elevation of plasma cholesterol value in supplemented group may be due to impurity of linoleic acid. Moreover, the rats used in the experiment were not hypercholesterolmic. The slight elevation in deficient group (Table 12) from 65 mg/dl (control value) to 98 mg/dl after 3 months and gradual declination with the advancement of maturity may be lipid metabolism. The circulation cholesterol may be related to elevated circulating insulin levels. Chobanian A. V. et al 1962, Inkeles et al 1981, Harris R. A. et al 1982. According to them decrease in insulin level associated with fasting, exercise between meals could favour reduced serum cholesterol as observed in the deficient group in present observation. Hegsted et al 1957 have suggested that
essential fatty acids present in unsaturated fat perhaps act together with the saturated fatty acids in producing more serum cholesterol values in experimental rats. Keys and associates 1959 found that in man slightly more than 2 gm of linoleic acid counter acts the effect of 1 gm saturated fatty acids in increasing serum cholesterol. On the basis of the findings of many workers on this line and the result of the present experiment, it may be suggested that the male rats require some amount of unsaturated fat in the diet to aid in the regulations of the cholesterol in the blood and tissues. From the literature it has been found that diets low in fat cause a decrease in the level of plasma cholesterol as found in the present observation, but there may be hypercholesterolemia in rats on the unsaturated fat free group (Borner and Day 1953) and cholesterol will be deposited in the liver; adrenal glands and other organs. Therefore, unsaturated fats may be required in the diet of rat for the metabolism of cholesterol in the blood because cholesterol metabolism and unsaturated fat are closely interrelated. Page I. H. 1956, Quackenbush F. W. 1960, Huang Y. S. et al 1987).

Blood plasma consists of an aqueous solution of protein which plays a vital role in different biological processes. Food proteins are
digested to aminoacids in the gastrointestinal tract and then pass into the portal blood stream. Protein level in blood is determined by the balance between the rates of addition and removal of aminoacids from the pool. In the present investigation, observation of plasma protein shows a steady level in both supplement and deficient groups of animals. There was no such elevation of declination in the plasma protein level which indicates that there was no destruction of liver or renal failure. But proteins of vegetable origin are deficient in one or more of the essential aminoacids. Bangal gram which is the only protein source in the present experiment is devoid of tryptophan and methinine, which may be responsible for the equal trend of elevation and depression of the metabolic activities of the liver tissue for production of plasma protein level.

The reason of large increase in serum alkaline phosphatase activity in deficient group may be due to absence of unsaturated fat in the diet. The basal level of the alkaline phosphatase in rat liver though very low, can be inducible by various nutritional factors of plant origin (Schimke et al 1970, Mildvan a. S. 1974). Large increase in alkaline phosphatase activity (Table No. 19) in serum in deficient group may not be due to unsaturated fat only
but may be due to biliary obstruction in the liver in the present investigation. Many authors have described that a greatly increased serum alkaline phosphatase activity in rats as observed in the present investigation results from administration of plant based nutritional products. However an increased alkaline phosphatase activity is also an indicator of biliary obstruction.

There was a elevation in serum alkaline phosphatase in supplement group as observed in the present investigation which may be due to the supplement of safflower oil which is of vegetable origin. This is also supported by many investigators Koshland D. E. Jr et al 1968, Mildvan A. S. 1974 that the plant based products may induce alkaline phosphatase activity in rats. The death of two animals of deficient group during the investigation may be due to the biliary obstruction. In biliary tract obstruction at any level may lead to the synthesis of alkaline phosphatase in the hepatocytes membrane, much of which escapes into the blood. A greatly increased serum alkaline phosphatase activity is therefore, the main indicator of biliary obstruction. Some times a raised plasma alkaline phosphatase activity is found incidentally and it may be cause of sole abnormality.
Taking into view all the observations of the present study, the cited literature and the data presented here in the present investigation, correlation co-efficient was calculated between all the observations of different parameters for all experimental groups. correlation coefficient was calculated in the control, deficient and supplement group between the parameters as follows -

2. Growth and plasma glucose.
3. Growth and plasma cholesterol.
4. Plasma glucose and plasma cholesterol.
5. Plasma glucose and plasma protein.
6. Plasma glucose and serum alkaline phosphatase.
7. Plasma cholesterol and plasma proteins.
8. Plasma cholesterol and serum alkaline phosphatase.

In the control group, a significant negative correlation, \( r = -0.09 \) was observed between growth and plasma protein. No significant correlation was found in any of the other tested groups though it may be mentioned that glucose and alkaline phosphatase showed \( \text{'r'} \) value of 0.43, growth and serum alkaline phosphatase had \( \text{'r'} \) value of 0.59, cholesterol and protein had \( \text{'r'} \) value of -0.25.
In the deficient groups for 12 months no significant correlation was found between growth and plasma protein with obtained 'r' value being 0.03. It means that negative correlation which existed between these groups in control was not established, when the rats were fed deficient of unsaturated fat diet for a long time. All the other correlation coefficient 'r' values obtained were not significant being more or less near to zero. Plasma glucose and serum alkaline phosphatase showed \( r = 0.24 \), growth and serum alkaline phosphatase \( r = -0.41 \), plasma cholesterol and serum alkaline phosphatase showed \( r = -0.59 \).

In unsaturated fat supplement group for 12 months, there was no significant correlation between growth and plasma protein with a 'r' value of 0.22. But there was a significant positive correlation \( (r = 0.80) \) with 5% chance of being wrong. When growth and plasma glucose were compared. Growth and plasma cholesterol \( (r = 0.65) \) and plasma glucose and plasma cholesterol \( (r = 0.64) \) also showed a positive correlation which cannot be said to be significant. No significant correlation is found to exist between the other tested groups, because all of them have 'r' values more or less near to zero.
FIG. XVIII SHOWING GROWTH (BODY WEIGHT) PLASMA GLUCOSE, PLASMA CHOLESTEROL, PLASMA PROTEIN AND SERUM ALKALINE PHOSPHATASE LEVELS IN DEFICIENT GROUPS; VALUES ARE EXPRESSED IN PERCENTAGES TAKING THE VALUES OF CONTROL ANIMALS AS 100 PERCENT.
FIG.: XIX. SHOWING GROWTH (BODY WEIGHT) PLASMA GLUCOSE, PLASMA CHOLESTEROL, PLASMA PROTEIN AND SERUM ALKALINE PHOSPHATASE LEVELS IN SUPPLEMENT GROUPS; VALUES ARE EXPRESSED IN PERCENTAGES TAKING THE VALUES OF CONTROL ANIMALS AS 100 PERCENT.
It was also observed in the investigation of the present study the serum alkaline phosphatase shows highest activity in the deficient group which is followed by the plasma glucose concentration (Fig XVIII) in relation to the control value. In the supplement group (Fig XIX) also serum alkaline phosphatase level shows higher elevation then any other biochemical factors as observed in the experiment. (Fig XVIII) and (Fig XIX) shows the relationships of all observed values in the present experiment in relation to the control value.

In the present study the effects of unsaturated fat diet on male albino rat, on all the above observations and after the comparative study with deficient and control group of animals, it can be suggested fat in the diet of rat is essential for the growth normal metabolic as well as structural state of organs like liver and kidney and cytochemical organizations. Biochemical study on plasma glucose, plasma cholesterol, plasma protein and serum alkaline phosphatase activity also show the essentiality of the unsaturated fat in the diet of rat. Although it is very difficult to point out the exact mechanism involved which brought about the observed changes in the line of morphological, histopathological, histochemical and biochemical points of view from the
foregoing discussion, it is apparent that the unsaturated fat in the diet acts as an essential nutrient supplement to bring about better growth, good health and efficient metabolic activities. For a more precise view on the problem expanded works involving more detailed and minute studies related with the problem are to be awaited.