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OF TWO HEPATIC ENZYME SYSTEMS.

Iowa State University of Science and Technology
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EFFECT OF DIETARY PROTEINS ON ACTIVITIES OF TWO HEPATIC ENZYME SYSTEMS

by

Mary Addina Crenshaw

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
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DOCTOR OF PHILOSOPHY

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1962

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INTRODUCTION

Research has shown that dietary protein functions in the regulation of the amount of hepatic fat deposited by experimental animals. Alterations in hepatic fat have been reported in response to modifications in the amount of dietary protein and to the addition or omission of a single amino acid (Harper et al., 1954a, Harper, 1958). The maintenance of experimental animals on a ration low in protein but adequate in all other known nutrients has been associated with a transitory rise in the concentration of hepatic fat which gradually fell as the growth rate declined (Harper et al., 1953a).

Intakes of protein inadequate to meet the needs of the body may also be accompanied by a reduction in enzymic activity as a result of decreases in the synthesis of enzymes or increased catabolism of enzymic protein to compensate for dietary deficiencies (Waterlow, 1959, Knox et al., 1956). When Waterlow and Patrick (1954) studied infants with fatty livers, they observed that the activities in vitro of hepatic succinoxidase, cytochrome reductase and the transaminases varied from the activities of the same enzymes in the livers of normal infants. Burch et al. (1957) reported reductions in concentrations of hepatic fat in patients after treatment for kwashiorkor. The activities of some hepatic enzymes also changed with treatment but the enzymes assayed did not include

those that contribute directly to the synthesis or catabolism of fat. In studies of the control of hepatic fat in experimental animals, the enzymic activities measured have also not been those directly related to the metabolism of fatty acids.

The pattern of enzymic activity has been used to evaluate the adequacy of dietary protein (Miller, 1950, Wainio _et al._, 1953). Use of low-protein and protein-free diets with experimental animals has shown that enzymic activities differ in their response to altered protein intakes. The activity of enzymes such as xanthine oxidase has decreased rapidly while a more gradual decrease has been observed with cytochrome oxidase and the transaminases as a result of reduced dietary protein. The enzymes assayed in such studies have not included the fatty acid oxidase (FAO) system which functions in the catabolism of fatty acids nor the hexose monophosphate (HMP) shunt dehydrogenases which can supply reduced triphosphopyridine nucleotide (TPNH) for the synthesis of fatty acids (Langdon, 1955).

The present experiment was planned to investigate the response of the FAO system and the glucose-6-phosphate (G-6-P) and 6-phosphogluconate (6-P-G) dehydrogenases in the livers of rats fed low intakes of casein or wheat gluten. Supplementation of each protein with its most-limiting amino acid was included to determine whether the addition of a single amino acid would produce any change in response.

From results of preliminary experiments and from reports in the literature a wider range of mean hepatic fat values was anticipated among the experimental groups than was obtained (Harper _et al_., 1954a). A wider range of hepatic fat would have permitted investigation of a possible three-way relationship between 1) intakes of protein, 2) activities of two enzyme systems, one of which participates in the synthesis, the other in the catabolism of fatty acids and 3) concentrations of hepatic fat associated with changing patterns of enzymic activity. However, some inferences have been drawn from comparisons of a control group fed stock ration with experimental groups since hepatic fat was increased significantly in the experimental groups compared with the controls.

REVIEW OF LITERATURE

The importance of dietary protein in the regulation of the concentration of hepatic fat and in the alteration of the activity of hepatic enzymes has been demonstrated repeatedly by the use of experimental animals maintained on controlled dietary rations. The activity of hepatic enzymes and the concentration of hepatic fat have also been investigated in protein deficiency in man and animals and in experimentally induced pathological conditions in animals.

The literature reviewed here includes reports of patterns of enzymatic activity in rats, reports of two enzyme systems which may be related to fat metabolism and reports of the influence of dietary factors on hepatic fat.

Activities of Hepatic Enzymes

General

Wainio *et al*. (1953) determined the rate of activity of a group of enzymes in the livers of rats after a period of protein depletion. The reduced activity of xanthine oxidase per milligram of hepatic nitrogen indicated a more rapid loss of enzymic protein than of total hepatic protein. A slower reduction in the rate of activity of cytochrome oxidase than of xanthine oxidase was observed among the same animals. These findings were interpreted to indicate the preferential

metabolism or preservation of specific hepatic enzymes occurred during protein depletion.

Knox *et al*. (1956) investigated responses of hepatic enzymes in rats subjected to fasting, to protein depletion and to high and low dietary intakes of protein. Of the hepatic enzymes which were measured, xanthine oxidase appeared to be one of the most sensitive enzymes in response to changes in dietary protein.

Hepatic xanthine oxidase activity in animals fed a purified diet providing protein and riboflavin adequate for growth was approximately 75% of that obtained in animals fed a stock ration (Westerfield and Richert, 1954). The factor or factors responsible for this difference in response to purified and stock rations were not identified.

Groups of weanling and adult rats were fed a 2% casein diet for three months after which period some of the animals from each group were realimented with a 20% casein ration while the remainder were kept on the 2% casein ration (Srinivasan and Patwardhan, 1955). *In vitro* determinations of the activity of several hepatic enzymes made at intervals during the period of depletion and realimentation indicated a much more rapid response to dietary deficiencies and a greater magnitude of alteration in activity among the growing animals than was observed in mature animals.

Ross and Batt (1957) were able to show a definite diet-

age pattern for the activities of hepatic histidase and alkaline phosphatase when the dietary protein and carbohydrate intakes were controlled in experimental animals. Proportions of the two nutrients could be adjusted to produce enzymatic activities in mature animals similar to those found in younger animals. Ross (1959) reported measurements of 23 hepatic enzymes during the first year of life of rats maintained on a stock ration. The activities of 18 enzymes increased with age, activities of four decreased and the activity of one enzyme remained unaltered. In the same study, protein depletion in rats of different ages resulted in a decrease in enzymic activity which corresponded to rates found in younger animals. The relative amounts of protein or carbohydrate in the ration appeared to exert a greater influence over enzymatic activity than did the caloric value of the food consumed.

Studies of enzymes in human subjects have been reported by workers who were attempting to determine the extent of the effects of malnutrition on patients who had applied for medical treatment. These patients served as their own controls. Enzymatic activities determined on initial contact with the patient were used as the basis for comparison with those obtained after treatment. Waterlow and Patrick (1954) reported decreased activity of succinoxidase and increased transaminase activities in the livers of infants from 6 months to two years

of age when the initial biopsies of the livers were made. Fatty livers were present in all subjects. However, these investigators suggested that the enzymic changes observed in these infants were not related to the fatty liver *per se*. The enzymes investigated in this and in other studies appear to have been chosen because techniques for assay were available rather than because of any direct relationship of their activities to metabolism of hepatic fat.

Oxidation of fatty acids

Most of the studies of the fatty acid oxidase system found in the literature were reports of assay techniques rather than investigations of functions of the system (Lehninger and Kennedy, 1948, Schneider, 1948). Artrom (1959) found that feeding ethionine to mature female rats inhibited the oxidation of fatty acids and resulted in an increased deposition of fat in the livers of these animals. These findings suggested that normal activity of the FAO system was necessary to protect the liver from fatty infiltration.

Synthesis of fatty acids

Activities of the G-6-P and 6-P-G dehydrogenases have been studied by varying the kind of dietary carbohydrate fed to normal animals and to those with experimentally induced diabetes (Fitch *et al*., 1959, Fitch and Chaikoff, 1960) and

by force-feeding diets to rats (Cohn and Joseph, 1959).

The rate of production of reduced triphosphopyridine nucleotide (TPNH) is a measure of the activity of the dehydrogenases. As a result of experimental work in which the disappearance of TPNH followed the addition of crotonyl coenzyme A(CoA) to a preparation of rat liver, Langdon (1955) suggested a possible requirement of TPNH as an electron donor in the biological synthesis of fatty acids.

When Tepperman and Tepperman (1958) fasted animals for 48 hours and refed them with a high carbohydrate diet, an increase in both hepatic fat and the activity of the shunt dehydrogenases over that determined before the period of fasting was observed. Hence these workers postulated that an increase in the activity of the hexose monophosphate (HMP) shunt followed an increased dietary intake of carbohydrate in the normal animal and the accelerated activity of this pathway of carbohydrate metabolism provided an increased quantity of TPNH which may have been used for lipogenesis. Conversely in the diabetic animal in the presence of decreased activity of the shunt dehydrogenases, a reduced or limited amount of TPNH was available and lipogenesis was retarded.

Hubbard _et al_. (1961) reported findings which suggested that the impaired lipogenesis associated with a reduced activity of the shunt enzymes might be determined by the concomitantly reduced activity of the enzyme system for the

synthesis of fat rather than by a reduced supply of TPNH.

Deposition of Hepatic Fat

Amount of dietary protein

Investigations have shown that the quantity of dietary protein can alter the amount of fat deposited in the liver of experimental animals. When proteins of relatively high biological value such as beef, pork, casein and egg albumin were fed to male weanling rats in amounts equal to or less than 9% of the dietary intake, an increase in hepatic fat was reported (Winje et al., 1954). The hepatic fat which accompanied such protein intakes varied from 10 to 15% on the wet weight basis. Increasing the quantity of any one of the proteins was followed by a reduction in the deposition of fat.

In an experiment with male weanling rats fed a diet containing 9% casein, 5% corn oil and sucrose as the dietary carbohydrate, hepatic fat was reduced by 50% after the addition of 2% casein or 6% gelatin (Harper et al., 1953a). The addition of 6% gelatin to a 9% casein diet containing choline and 0.3% methionine reduced hepatic fat from 13.7 to 4.6% of the wet weight of the liver (Harper et al., 1954d).

Beveridge et al. (1945) fed young rats experimental rations which contained 40% fat from beef drippings and were low in choline. Casein was used as the dietary protein and was fed in amounts varying from 10 to 40% of the ration. In the

experiments in which animals were fed these diets, the amount of hepatic fat was decreased markedly when casein was increased from 10 to 20% of the ration. Further reductions in the concentration of fat were obtained by increasing the casein to 40% of the diet.

Similar decreases in the concentration of hepatic fat with increases in wheat protein were reported by Harris and Burress (1959). Rats maintained on an 8% wheat protein diet developed a fatty liver syndrome which did not appear in animals fed 15% wheat protein.

There appears to be a relationship between the rate of growth and the quantity of protein required to produce or prevent the deposition of excessive amounts of fat in the liver. Increases in hepatic fat were obtained in weanling rats when 9% casein diets were used but a reduction to 5% casein was necessary to increase hepatic fat in mature animals (Harper et al., 1954c). When the 9% casein diet was fed for prolonged periods, hepatic fat was reduced as the rate of growth decreased (Harper et al., 1953b).

Supplementation of dietary protein with amino acids

Experiments designed to increase knowledge of the effects of amino acid imbalance have provided additional information about production and prevention of fatty livers. The amount of fat deposited in the liver is a criterion that has been

used in evaluating the balance or imbalance of amino acids produced when experimental animals are fed small amounts of protein supplemented with one or more amino acids.

Harper et al. (1953a) fed a basal diet which consisted of 9% casein, 5% corn oil and sucrose as the dietary carbohydrate. Supplementation of this ration with 0.18% threonine resulted in a reduction of hepatic fat in male weanling rats from 7.6-9.6% to 3.5% on the wet weight basis. The addition of either glycine or serine as single amino acid supplements to the basal ration also proved beneficial in reducing accumulations of hepatic fat. Other nitrogen-containing compounds were tested, all of which could be metabolized to glycine and thence to serine. Because beneficial effects resulted from this group of supplements it was suggested that hydroxy amino acids might participate directly in utilization of fat.

Winje et al. (1954) reported the effectiveness of threonine as a supplement to a 9% casein diet but found threonine to be less effective in reducing concentrations of hepatic fat when smaller amounts of casein were fed. Threonine was found to be a more effective supplement with casein and beef than with pork or albumin. The fact that threonine was not equally effective with all proteins indicated that other amino acids have a role in the regulation of hepatic fat. These investigators suggested that a specific ratio of amino acids might be required to maintain a normal concentration of fat in

the liver.

Deshpande et al. (1958a) found that supplementation of 9% protein diets with methionine and tryptophan precipitated a threonine deficiency characterized by an increase in hepatic fat to 25 to 30% of the wet liver weight. This deficiency was observed when beef, casein and egg albumin were tested but was not detected when a 6% fibrin diet was fed. The increase of tryptophan and methionine stimulated the growth rate which in turn apparently increased the requirement for threonine. The feeding of the 6% fibrin diet with supplements of methionine and threonine resulted in a poor rate of growth which may explain the reduced need for threonine and the absence of fatty livers in these animals.

Harris and Burress (1959) demonstrated that the fatty liver produced in weanling rats fed an 8% wheat protein diet could be prevented by supplementation of the diet with lysine. Since the increase in the concentration of hepatic fat was not observed when the diet contained 15% protein it was suggested that the deficiency of lysine which is the most-limiting amino acid in the wheat protein does not become apparent unless the protein is fed in small amounts.

Harper et al. (1954b) described an anti-lipotropic effect of methionine when used to supplement a 9% casein diet in which sucrose was used as the source of carbohydrate. The effect was not obtained when other proteins replaced casein in

this ration or when the casein level was increased to 15%. The increased accumulation of hepatic fat produced by the supplementation of the 9% casein-sucrose diet with methionine appeared to result from a partial deficiency of threonine which was precipitated by the addition of methionine. Supplements of 0.36% threonine were used successfully to reduce hepatic fat from 9.7 to 4.2% of the wet weight of the liver in mature rats maintained on the casein-sucrose ration supplemented with 0.3% methionine (Harper et al., 1954c).

Dietary carbohydrate

Harper et al. (1953b) used a basal ration containing 9% casein to test the effects of dextrin, cerelose, lactose, fructose and sucrose on the deposition of hepatic fat and on growth rates in weanling rats. Sucrose appeared to produce the greatest accumulation of fat and dextrin the smallest, although the growth rate of the animals receiving dextrin was two to three times that of the animals receiving sucrose. The other dietary carbohydrates were intermediate in their effect on growth and hepatic fat. An increase in the amount of dietary casein resulted in a decrease in the deposition of fat with all carbohydrates.

Marshall and Womack (1954) studied differences in the accumulation of fat in the livers of experimental animals when corn dextrin or sucrose was used as the dietary carbohydrate.

They found less fat in the livers of animals receiving corn dextrin than in animals receiving sucrose when other dietary components remained constant.

Dietary fat

The deposition of fat in the liver is not dependent on the presence of fat in the diet. Animals on a fat-free diet exhibited slight elevations in hepatic fat when compared with those receiving the same ration to which 5% corn oil had been added (Harper et al., 1954a).

Influences of dietary fat on accumulations of fat in the liver of experimental animals are closely related to both protein intake and kind of fat. The use of 5% corn oil or margarine in the 9% casein-sucrose diet resulted in lower fat in the liver than did the use of the same amount of butter fat or lard. The addition of threonine to the ration reduced the hepatic fat resulting from each of the four dietary fats but did not alter the comparative differences produced by corn oil or margarine and those produced by butter fat or lard. An increase in corn oil from 5 to 15% in the 9% casein-sucrose basal ration produced no increase in fat in the livers of weanling rats (Benton et al., 1956).

A reduction of casein from 9% to 7% of the diet resulted in increased differences between amounts of hepatic fat associated with corn oil and butter fat. The magnitude of

difference in fat accumulation in the liver was further increased by the use of a 9% casein diet which was deficient in choline. From this work it would appear that adequate amounts of dietary protein and of choline are important factors in preventing the accumulation of fat in the liver of experimental animals fed fats which include long-chain saturated fatty acids.

Other dietary factors

Best et al. (1954) summarized substances recognized as lipotropic factors and restated the criteria on which their recognition is based. Choline, betaine and methionine were included in the list of lipotropic factors while inositol and vitamin B_{12} were included only as factors sometimes known to function in a lipotropic capacity. The quantity of each of these dietary components required to prevent the accumulation of hepatic fat is dependent on the protein of the diet. If dietary protein is adequate in quantity and in quality, methionine may be used for synthesis of choline and hence the requirement for choline may be reduced. When a protein-deficient diet which was adequate in choline was fed to mature rats, Koch-Weser et al. (1953) were unable to prevent the development of fatty livers in these animals even when the amount of choline was increased hence demonstrating a relationship between dietary protein and the amount of choline

required to prevent the accumulation of hepatic fat. Griffith and Wade (1939) found it necessary to provide more choline in diets for weanling rats than for adult rats in order to protect the liver from fatty infiltration. The lipotropic factors together with adequate dietary protein protect the liver from excessive accumulations of fat by reducing the deposition or synthesis of hepatic lipid or by increasing the rate of removal of fat from the liver.

Scrimshaw et al. (1958) attributed fatty liver found in kwashiorkor to an excess of food energy in relation to protein. No increase in hepatic lipid was found where food intakes were reduced. Yoshida et al. (1961) also stressed the importance of a balance between the caloric intake and the amount of dietary protein in preventing the accumulation of hepatic fat in experimental animals. These workers were able to prevent the development of fatty livers in weanling rats on a threonine-deficient diet by a moderate restriction of food intake, although no retardation of growth was observed. The food intake on the restricted diet amounted to 70% of the ad libitum intake.

METHOD OF PROCEDURE

General Plan of Study

Two proteins were fed as 9% of the diet to each of two groups of male weanling rats for three weeks. Diets containing each of the dietary proteins were supplemented with the most-limiting amino acid and adjusted so that all diets were isonitrogenous. The two supplemented diets were fed to two additional groups of animals. A fifth group of animals was maintained on the laboratory stock ration and used as the control group. Intakes of food and changes in weight were recorded for each animal. After a 24 hour interval of fasting the animals were sacrificed and the liver was removed and divided into three weighed portions. The concentration of fat and the rates of activity of FAO and the HMP shunt dehydrogenases were determined in the livers of each animal.

Experimental Animals

Male rats of Wistar stock were weaned at the age of 28 days. Those weighing between 55 and 75 gm were assigned in rotation to each of 5 dietary treatments until a total of 5 animals had been obtained for each treatment. These 25 animals were maintained on the diets during the winter months and will be referred to as Group A. Groups B and C were chosen in the same manner and consisted of 6 animals on each

of the 5 diets. Groups B and C were maintained on the diets during the spring and summer months respectively. Hence a final total of 17 animals was obtained for each dietary treatment. Animals were housed individually and intakes of food and weights of the animals were recorded three times per week.

All animals were fasted 24 hours prior to sacrifice. Animals were sacrificed on the 20th, 21st or 22nd day of the experimental period by stunning and decapitation. The liver was removed immediately and divided into three weighed portions. Two samples weighing approximately 200 to 500 mg each were taken from the tri-lobate section for determinations of enzyme activity. One sample was assayed immediately for FAO activity; a second sample was wrapped in aluminum foil and stored in a freezing compartment of a refrigerator for 20 to 30 minutes until the determination of the activity of the HMP shunt dehydrogenases was begun. The remainder of the liver was wrapped in aluminum foil and stored in a freezer for a later determination of the concentration of hepatic fat.

Experimental Diets

The laboratory stock diet (Table 19, page 95) which was fed to the group of control animals was supplemented with 5 gm of ground raw lean beef fed three times per week, 10 gm of raw carrots fed twice and 10 gm of raw cabbage fed once each week. Two drops of cod liver oil were given three times per week and

two drops of a solution of alpha-tocopherol in Wesson oil which supplied 3 mg of alpha-tocopherol were given once each week.

The composition of each of the experimental diets is given in Table 1. These diets were supplemented with 2 ml of a solution of crystalline vitamins in 20% ethanol given three times weekly together with two drops of cod liver oil. The composition of the vitamin mixture is given in Table 2. Two drops of the alpha-tocopherol solution were added to the vitamin supplement once each week.

The experimental diets were prepared in lots of 1500 gm. The dry dietary components were sifted together twice to insure even mixing. The fat was melted and rubbed into the dry mixture with the fingers. This granular mixture was then forced through a screen and remixed. Each of the crystalline amino acids was combined with a portion of dextrin by grinding in a mortar with a pestle. The dextrin-amino acid mixture was then sifted three times with the remaining dry components of the diet before the melted fat was added. All diets were stored in small covered containers in a freezer, approximately 250 gm of the mixture being removed to a refrigerator for daily feedings.

Analytical Procedures

Fatty acid oxidase system

The fatty acid oxidase system is found in the mitochondria (Schneider, 1948, Kennedy and Lehninger, 1950) and func-

Table 1. Composition of experimental diets in grams per 1000 grams of experimental diet

Dietary components	Casein I	Casein II	Wheat Gluten I	Wheat Gluten II
Casein	108.0	105.0	--	--
Wheat gluten	--	--	112.0	109.0
DL-methionine	--	3.0	--	--
L-lysine	--	--	--	3.0
Dextrin	652.0	652.0	648.0	648.0
Crisco	200.0	200.0	200.0	200.0
Hawk and Oser salts	40.0	40.0	40.0	40.0
% of diet as protein	8.98	9.04	8.99	9.05
% of calories as protein	7.55	7.34	7.58	7.37

Table 2. Composition of vitamin supplement

Vitamin	Mg
Thiamine	47
Riboflavin	93
Pyridoxine-hydrochloride	47
Folic acid	47
Calcium pantothenate	233
Para-amino benzoic acid	233
B_{12}	470 of 0.1% in mannitol
Biotin	470 of 1.0% in dextrin
Niacin	154
Inositol	5,800
Choline	11,600
Ethanol, 20%, added to make to 2000 ml	

tions by the removal of two-carbon fragments from fatty acids (Green and Mii, 1953). A free carboxyl is required for function of the system. Magnesium ions, adenosine triphosphate (ATP) and a "priming" metabolite such as succinate or malate must be added to the system for determinations _in vitro_ (Lehninger and Kennedy, 1948, Deuel, 1957, Mehler, 1957). The optimum pH is 7.4. Sucrose is required to maintain the integrity of the mitochondria (Hogeboom _et al._, 1948). Substrates used for measurements _in vitro_ of activity included oleate,

which is similar to fatty acids present for the system *in vivo* but is not readily soluble, and octanoate, which has been widely used because of its solubility (Greenbaum and McLean, 1953).

The rate of activity of the FAO system was measured manometrically and recorded as the rate of molecular oxygen uptake resulting from the oxidation of a measured amount of octanoic acid by 50 mg of liver. The analytical method used was that of Greenbaum and McLean (1953). The temperature for running the assay was reduced from 37°C to 32°C in order to obtain a straight-line uptake of oxygen for more than 10 minutes.

The estimate of enzymic activity represents an average of the difference between the corrected triplicate determinations of samples and blanks. The oxygen uptake at 32°C remained linear throughout 20 minutes. However, data obtained from the line at the 10 minute interval were used as the basis for the computations reported in this thesis.

The reagents for FAO determinations and their desired molarities in the total 3 ml volume added to each Warburg flask are listed in Table 3. All measurements were made from solutions which had been equilibrated to room temperature. The mixture of reagents listed in Table 3 was prepared immediately before the animal was sacrificed and was adjusted to pH 7.4. Aliquots of 2.1 ml of the mixture of reagents were

Table 3. Desired molarities of reagents and amounts of reagents used for a single determination of the activity of the FAO system

Reagent	Desired molarity	Amounts used per 3 ml volume		Combination of reagents prepared for blank and sample[a,b] (ml)
		Sample (ml)	Blank (ml)	
Na_3PO_4 buffer	1.00×10^{-2}	0.3	0.3	2
KCl	2.50×10^{-2}	0.3	0.3	2
$MgCl_2$	6.60×10^{-3}	0.3	0.3	2
ATP	2.00×10^{-3}	0.6	0.6	4
Succinate	1.00×10^{-3}	0.3	0.3	2
Cytochrome C	2.66×10^{-5}	0.3	0.3	2
Octanoate	1.33×10^{-3}	0.3	--	
Deionized water		--	0.3	
Liver homogenate		0.6	0.6	

[a]Pool from which 2.1 ml were withdrawn for each determination.

[b]pH of pool was adjusted to 7.4 by addition of 3 to 5 drops of approximately 0.13N KOH.

withdrawn from this pool and placed in each of 6 Warburg flasks. A 0.3 ml portion of octanoic acid solution was added to each of three flasks for the sample determinations and an equal volume of deionized water was added to the three flasks for the blank determinations.

The animal was sacrificed immediately following the preparation of the reaction flasks. A sample of liver weighing between 350 and 500 mg was added to an ice cold solution of 0.25M sucrose to give a concentration of 50 mg of liver per 0.6 ml. The sucrose-liver mixture was homogenized for 40 seconds in an ice bath using a motor-driven glass homogenizer with a "Teflon" pestle.

Aliquots of 0.6 ml of the homogenate were added to the reagents in the 6 reaction flasks within 7 to 9 minutes after the animal was sacrificed. At the end of an equilibration period of 5 minutes the manometers were adjusted to 150 mm and the stopcocks closed. Oxygen uptake was read at 5 minute intervals for 20 minutes.

<u>Activities of the G-6-P and 6-P-G dehydrogenases</u>

The G-6-P and 6-P-G dehydrogenases participate in the metabolism of carbohydrate via the hexose-monophosphate shunt pathway. These cytoplasmic enzymes are found in the supernatant when homogenized liver is fractionated by centrifugation. The combined rate of activity of the two dehydro-

genases was determined spectrophotometrically by measuring the rate of change in optical density at 340 millimicrons produced by the reduction of TPN by 5 mg of liver at pH 7.6. The method used was that of Glock and McLean (1953). The reagents for determination were combined in the proportions recommended in a communication from the Sigma Chemical Company.[1]

A sample of liver which had been refrigerated not longer than 30 minutes was homogenized in 8 volumes of cold 0.15M KCl solution to which $KHCO_3$ had been added to obtain a pH 7.0. The mixture was homogenized for 40 seconds in an ice bath using a motor driven glass homogenizer with a "Teflon" pestle.

A 0.4 ml aliquot of the homogenate was added to a 23.6 ml volume of reagents for the sample and 0.15 ml of homogenate was added to 8.85 ml of reagents for the blank determination as described in Table 4. Following the addition of the homogenate the mixtures were agitated constantly. A 2.5 ml aliquot was withdrawn from the sample mixture at one minute intervals for 6 minutes following the addition of the homogenate. Each of these aliquots was placed in a stoppered centrifuge tube which contained 2.5 ml of 95% ethanol and 0.1 ml of 10% Na_2SO_4. Samples of the mixture for the blank determinations were withdrawn at the end of the first and sixth

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[1]Sigma Chemical Company, Saint Louis, Missouri. Assay of glucose-6-phosphate dehydrogenases. Private communication. July 10, 1959.

Table 4. Desired molarities of reagents and amounts of reagents used in mixtures for determinations of the activity of the G-6-P and 6-P-G dehydrogenases

Reagent	Desired molarity	Amounts of reagents used in mixture[a] Sample (ml)	Blank (ml)
$MgCl_2$	0.10	1.6	0.60
Glycyl-glycine	0.25	4.0	1.50
Glucose-6-phosphate	0.02	2.4	0.90
TPN	0.001	2.0	--
Deionized water		13.6	5.85
Liver homogenate		0.4	0.15

[a]Mixture from which 2.5 ml were withdrawn for each determination.

minutes and added to centrifuge tubes prepared as for the samples.

Samples and blanks were allowed to stand for 30 minutes to precipitate the protein. The tubes were then centrifuged 5 minutes and the clear supernatant was poured into 1 cm cuvettes. Readings were made on a Beckman Spectrophotometer at 340 millimicrons.

Concentration of hepatic fat

The liver that remained after the removal of samples for the determinations of the activities of the two enzyme systems was homogenized in distilled water and made to a volume of 50 ml. The fat in a 10 ml aliquot of homogenate was extracted in Mojonnier extraction flasks by a combination of ethyl and petroleum ethers (Soderhjelm and Soderhjelm, 1949). The ether extracts were poured into weighed containers and evaporated over a water bath. The weighed containers were placed in an oven at 85°F for 15 hours after which they were stored in a desiccator until they could be weighed at room temperature.

RESULTS AND DISCUSSION

Growth Index and Hepatic Fat Levels

Changes in weight during the three weeks of the experimental period have been expressed as a "growth index" which is the quotient obtained by dividing the final weight of the animal after three weeks on an experimental diet, and prior to the 24 hour fasting period, by the weight of the animal at weaning.

For discussion the animals are classified by the 5 diets on which they were maintained: Control, Casein I, Casein II, Wheat Gluten I and Wheat Gluten II. The control animals were fed the stock ration; Casein I, 9% casein; Casein II, 8.7% casein plus 0.3% methionine; Wheat Gluten I, 9% wheat gluten; and Wheat Gluten II, 8.7% wheat gluten plus 0.3% lysine.

Mean values for growth index, liver weight, concentration of hepatic fat and daily food consumption with the corresponding standard deviation for each computation are presented in Table 5.

The Control group fed the stock ration supplemented with natural foodstuffs and with cod liver oil attained a final mean weight which was 2.35 times that of the mean weight of the group at weaning. The growth indices for the individual animals within the group ranged from 2.11 to 2.75.

Diets containing 9% protein are inadequate to support

Table 5. Mean daily food intake, mean liver weight, mean growth index and mean hepatic fat concentration

Group	Number of animals	Mean daily food intake (gm)		Mean liver weight (gm)		Mean growth index		Mean hepatic fat concentration (%)	
Con.	17	--	--	4.61	±0.36	2.35	±0.02	2.58	±0.55
Cas. I	17	6.5	±0.6[a]	2.36	±0.25	1.42	±0.06	6.73	±2.07
Cas. II	17	7.6	±0.8	2.95	±0.25	1.78	±0.13	6.17	±1.39
W.G. I	17	4.7	±0.5	1.68	±0.11	0.98	±0.03	5.77	±1.26
W.G. II	17	5.6	±0.4	2.13	±0.19	1.16	±0.05	8.39[b]	±6.69

[a]Standard deviation.

[b]Median value for hepatic fat is 6.36.

optimal growth in the weanling rat even when the biological value of the protein is high (Mitchell, 1959). Animals maintained on unsupplemented 9% casein had a mean growth index of 1.42 with a range from 1.31 to 1.53 among the individual animals. The mean daily food intake was 6.5 gm for the group fed the unsupplemented casein diet and 7.6 gm for the group fed the Casein II diet. A mean growth index of 1.78 was observed in the Casein II group due to the increased food intake resulting from the inclusion of methionine, the most-limiting amino acid of casein for growth in the rat (Harper, 1959). Growth indices for the animals on the supplemented casein ration ranged from 1.52 to 1.93.

The use of diets containing 9% protein from wheat gluten was accompanied by a voluntary reduction in food consumption and a more severe retardation in growth than was observed when the dietary protein was casein. These observations were in keeping with those of Harper and Katayama (1953) who also reported reductions in food consumption and in growth when proteins of poor quality were fed in small amounts. Only 2 of 17 animals in the Wheat Gluten I group had gained weight at the end of the experimental period. Losses of from 1 to 4 gm were observed in 14 animals while the remaining animal maintained its weaning weight. Hence the mean growth index for the group was 0.98 with a range from 0.94 to 1.05 among the individual animals.

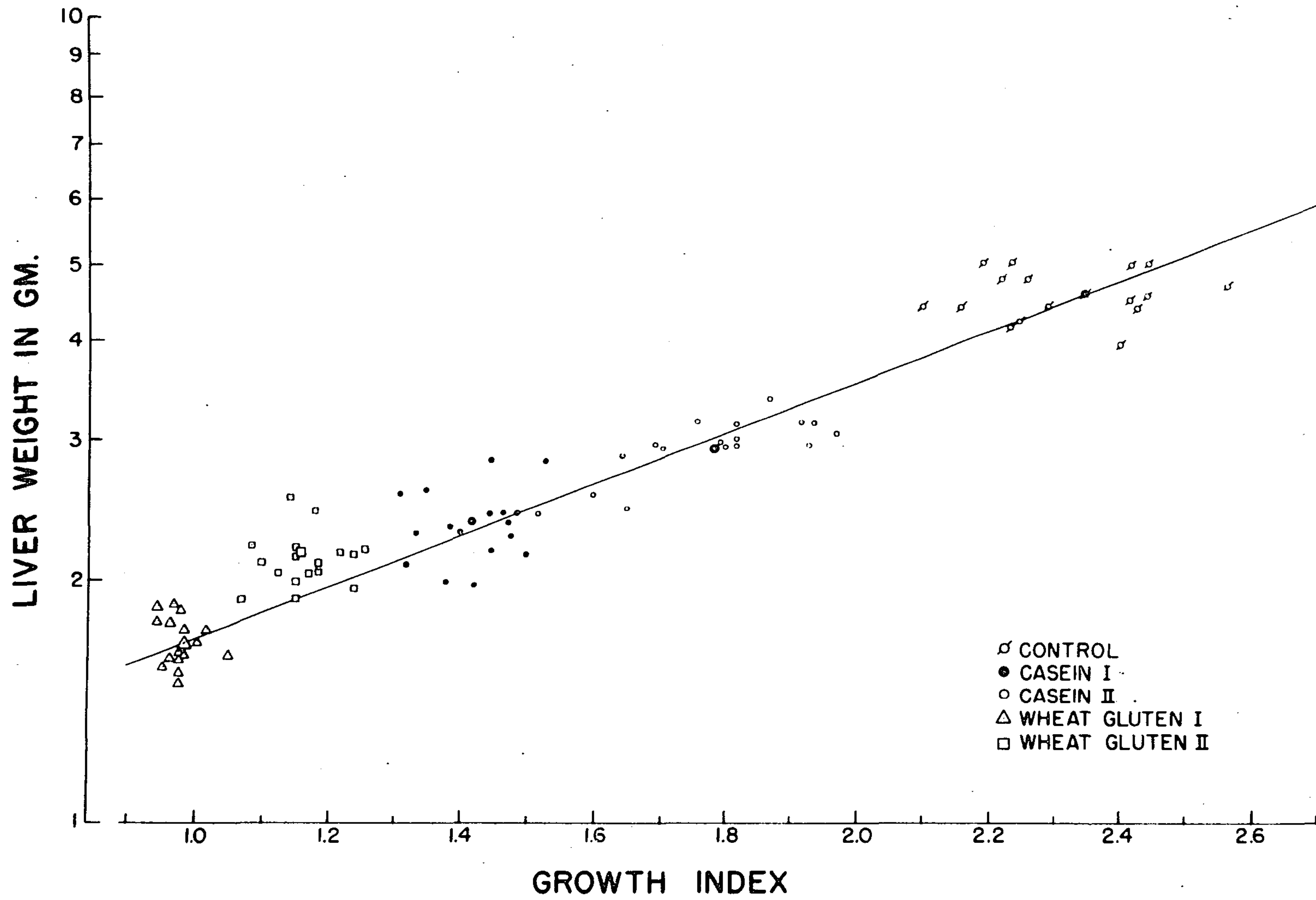
When the wheat gluten ration was supplemented with 0.3% lysine, growth was small although the mean daily consumption of food was 0.9 gm greater than the intake of the animals consuming the unsupplemented wheat gluten diet. Growth indices among the animals in the Wheat Gluten II group ranged from 1.07 to 1.26 with a mean growth index of 1.16 for the group.

Weights of livers within all groups appeared to increase with an increase in the growth index. A linear relationship was observed for data of individual animals within the control group, the two casein groups and the unsupplemented wheat gluten group when the logarithm of the liver weight was plotted against the growth index. The liver weights for the supplemented wheat gluten group lay slightly above the line which appeared to fit the plotted values for the three other experimental groups and the control animals (Figure 1). The livers averaged 3.5% of the fasted autopsy weight for the control group, 2.8% for Casein I, 2.8% for Casein II, 2.9% for Wheat Gluten I and 3.1% for Wheat Gluten II groups.

Values for hepatic fat have been expressed as percentages of the wet weight of the liver. Animals on the stock ration had a mean hepatic fat of 2.58% with values from individual livers ranging from 1.44 to 4.30%.

The mean value for hepatic fat for each of the 4 groups maintained on the purified diets was significantly higher than that observed for animals maintained on the stock ration.

Figure 1. Relationship of liver weight to growth index in control and experimental animals



LIVER WEIGHT IN GM.
10
9
8
7
6
5
4
3
2
1
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
GROWTH INDEX
CONTROL
CASEIN I
CASEIN II
WHEAT GLUTEN I
WHEAT GLUTEN II

Examination of values for individual animals in the Casein II, Wheat Gluten I and Wheat Gluten II groups, however, showed some overlap between these groups and the control group. Hepatic fat values among animals in the Casein I group ranged from 4.36 to 10.94% and did not overlap with values in the Control group.

The presence of 0.3% methionine in the Casein II diet resulted in a mean hepatic fat value of 6.16% compared with a mean value of 6.73% for the animals on the unsupplemented ration. Values among the Casein II animals ranged from 4.13 to 8.94% and were similar to the values observed among the Casein I animals. Therefore, the mean hepatic values for these two groups were not significantly different (Duncan, 1955).

The lipotropic effect of methionine is dependent on the amino acid pattern and the total protein content of the diet. Hepatic fat values of 13.7% on the wet weight basis were observed among weanling male rats fed a 9% casein-sucrose ration to which 0.3% methionine was added (Harper et al., 1954b). When methionine was increased to 1.0% of the diet a reduction in hepatic fat to 9.7% was observed. However, further increases in methionine supplementation of the 9% casein-sucrose diet did not reduce hepatic fat to concentrations found in animals maintained on stock rations.

In the present experiment the widest range of hepatic

fat, from 3.52 to 29.10%, was observed among individual animals receiving the ration which contained wheat gluten plus lysine. The mean value of 8.39% for this group was reduced to 7.10% when the highest value, 29.10%, was disregarded in the computations. However, use of the median value of 6.36% appeared to provide a parameter more nearly descriptive of the group. The range of hepatic fat concentrations in the unsupplemented wheat gluten group was from 3.32 to 8.14% with a mean hepatic fat of 5.77%.

In two preliminary experiments weanling rats were fed a 22% casein-9% Crisco diet for three weeks after which they were placed on diets similar to those reported here. In one experiment female rats fed 9% wheat gluten rations maintained their weight and a mean hepatic fat of 7% was reported for the group. In the same experiment animals which were fed 9% wheat gluten plus 0.3% lysine gained on the average 7 gm per week and their livers contained 13.4% fat.[1] In the second experiment male rats fed the 9% wheat gluten ration had a mean gain in weight of 0.6 gm per week and a mean hepatic fat concentration of 6.6%. Hepatic fat ranged from 4.2 to 8.6% among the individual animals in this group. Other male rats in the second experiment were fed a ration with 8.55% wheat gluten plus 0.45% lysine which resulted in a mean gain in weight of

[1]Farris, Dianne M. Ames, Iowa. Data from experiment. Private communication. 1959.

12 gm per week and a mean hepatic fat of 26%. Individual animals within the latter group had a range of hepatic fat from 9.7 to 43.0%.[2] In the present experiment the concentration of hepatic fat was considerably smaller than that observed in the preliminary experiment. The difference in the concentrations of fat between the present and preliminary experiments may have been influenced by the use of the 22% casein diet for three weeks before animals in the preliminary experiments were placed on the low protein ration. Hence animals in the preliminary experiments weighed considerably more at the time the low protein diet was introduced than did animals in the present experiment.

Contradictory responses to amino acid supplementation were observed when casein and wheat gluten were supplemented with their most-limiting amino acids in the present experiment. The addition of lysine to wheat gluten permitted weight gains which ranged from 5 to 17 gm among animals for the experimental period of 3 weeks. The mean deposition of lipids in the livers of this group of rats was significantly higher at P = 0.05 than that found in the other three experimental groups (Duncan, 1955). Supplementation of the casein diet with methionine was accompanied by an acceleration in growth and a decrease in mean hepatic fat from 6.73 to 6.16% which

[2] Buck, Virginia and Gibson, Katherine. Ames, Iowa. Data from experiment. Private communication. 1959.

was, however, not a significant difference at P = 0.05 (Duncan, 1955).

Deshpande et al. (1958a) fed a 9% casein-sucrose ration, supplemented with methionine and tryptophan, to young growing rats and reported increased accumulations of fat in the livers which these workers attributed to a threonine deficiency. From the results of the supplementation of the casein-sucrose ration with methionine and findings in an experiment in which animals were fed 6% fibrin with amino acid supplements, Deshpande et al. (1958b) suggested that the accelerated growth which accompanied amino acid supplementation of low protein diets required the utilization of additional amino acids for tissue synthesis and thereby reduced the amino acids available to protect the liver against fatty infiltration.

When the highest values of hepatic fat were analyzed for relationships to growth indices, 4 animals in the Wheat Gluten II group had hepatic fat concentrations that exceeded 10%. Three of these 4 animals had identical growth indices which exceeded the mean growth index for the group. This association of increased hepatic fat with increased growth indices is in keeping with the possibility of an increased demand for amino acids for growth, hence a decreased availability of amino acids for protection of the liver. Also, in the group maintained on the unsupplemented wheat gluten diet the lowest hepatic fat value was associated with the lowest growth index for the

group.

Fatty Acid Oxidase Activity and Hepatic Fat

Oxygen taken up due to the activity of the FAO system of 50 mg of liver was measured using octanoic acid as the substrate. When the corrected total volume of oxygen taken up at 5 minute intervals was plotted against time over a 20 minute period a linear relationship was obtained for both control and experimental groups. The uptake at 10 minutes as estimated from the straight line was used to compute activities of the FAO system expressed as microliters of oxygen per 50 mg of liver, per total liver and per 100 gm of body weight.

Other reports have also expressed the activities of enzymes on the basis of protein nitrogen and of desoxyribonucleic acid (Waterlow, 1952). The determination of hepatic nitrogen in the present experiment was not possible in addition to the determinations of hepatic fat and enzymic activities since the weights of the livers of 6 animals from the Wheat Gluten I group were 1.6 gm or less.

Total activity of the liver was discussed and used in experiments of Fitch and Chaikoff (1960). Weber (1959) reported similar changes in activities of enzymes involved in carbohydrate metabolism when such activities were expressed on the basis of per 100 gm of body weight or per average liver cell.

The mean values and corresponding standard deviations obtained for each method of expression in the present experiment are presented for each group of animals in Table 6. This table also includes the mean percentage of activity observed among the experimental groups when the mean activity of the Control group was assumed to be 100%.

When activities were expressed on each of the three bases, the control animals showed a significantly higher mean activity at $P = 0.05$ than did those groups receiving any one of the purified rations (Duncan, 1955).

Alterations in enzymic activities have been observed when supplies of dietary protein were less than optimal for growth, when purified diets were used and when the protein content of the liver was decreased in protein depletion or deficiency. Animals in the present experiment maintained on purified diets were subjected to amounts of protein that were less than optimal for growth and therefore, to the possibility of a decrease in hepatic protein. Hence several factors may have exerted an influence on the differences in FAO activity between control and experimental groups.

The adequacy of the stock ration fed to the Control group had been determined by analyses and was demonstrated by the superior rate of growth of animals maintained on it. The animals in the experimental groups received a suboptimal amount of dietary protein and those maintained on wheat gluten

Table 6. Mean activity of the FAO system per 50 mg of liver, per total liver and per 100 gm of body weight

		(Uptake of oxygen in microliters per 10 minutes)					
Group	Number of animals	Per 50 mg of liver (microliters)	(%)	Per total liver (microliters)	(%)	Per 100 gm of body weight (microliters)	(%)
Con.	16	50.30 ± 6.71[a]	100	4599 ± 690	100	3500 ±537	100
Cas. I	17	32.70 ± 5.78	65	1529 ± 234	33	1808 ±300	52
Cas. II	17	38.70 ± 5.33	77	2269 ± 272	49	2170 ±347	62
W.G. I	17	29.60 ± 3.52	59	993 ± 141	22	1726 ±201	49
W.G. II	17	36.40 ± 9.64	72	1533 ± 365	33	2209 ±553	63

[a]Standard deviation.

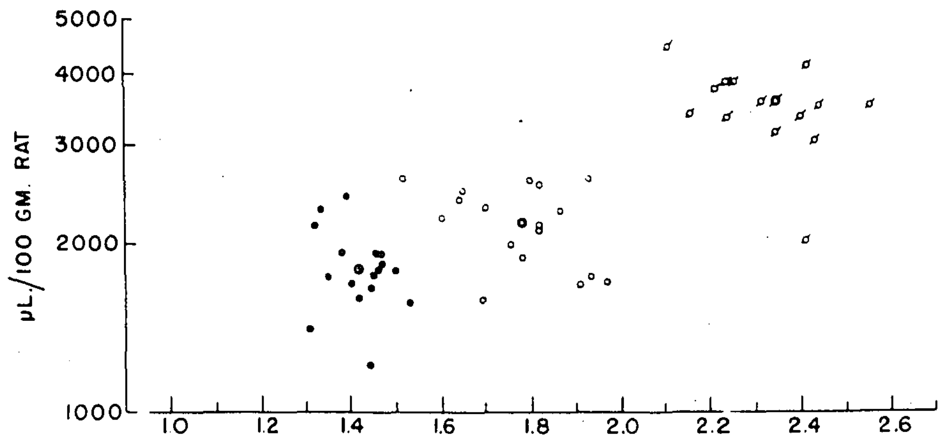
were further limited by the amino acid pattern of a cereal protein of relatively low biological value. Wainio <u>et al</u>. (1953) reported an experiment from their laboratory in which inadequate intakes of dietary protein resulted in a reduction in total hepatic protein accompanied by a decrease in the activity of a group of oxidative enzymes in the livers of mature rats.

When Westerfield and Richert (1954) used purified diets made as nearly adequate in all nutrients as possible they were unable to obtain xanthine oxidase activities greater than 75% of those found in animals on a stock ration. If the purified diets affect the activity of the FAO system in the same direction, higher activities would be predicted among animals on the stock ration than among those on the present experimental diets. This prediction held true in this experiment.

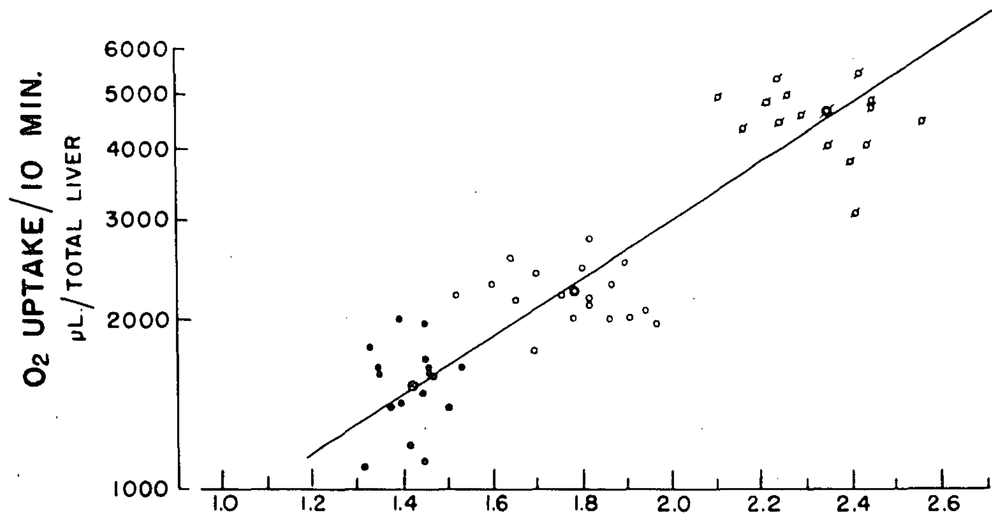
With the severe growth retardations imposed by the Wheat Gluten I diet and with the wide range of responses obtained within the Wheat Gluten II group, relationships were more readily examined by comparisons among animals in the Control, Casein I and Casein II groups considered separately from the groups maintained on the wheat gluten diets (Figure 2).

When the mean activity of the FAO system was expressed per unit of liver, per unit of body weight or per total liver the order of magnitude remained consistent with the highest

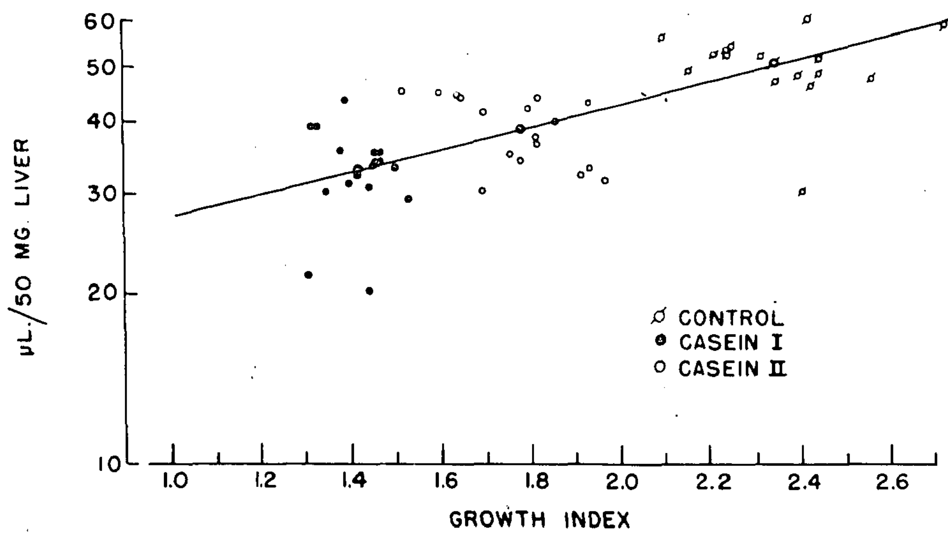
Figure 2. Relationship of FAO activity to growth indices in animals fed Control, Casein I and Casein II diets



5000
4000
3000
2000
1000
µL./100 GM. RAT
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6



O_2 UPTAKE/10 MIN.
6000
5000
4000
3000
2000
1000
µL./TOTAL LIVER
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6



60
50
40
30
20
10
µL./50 MG. LIVER
CONTROL
CASEIN I
CASEIN II
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
GROWTH INDEX

rate of activity in the Control group followed in descending order by the Casein II and Casein I groups (Table 6). The same order of relationship among these three groups was observed for mean growth index values but the inverse order was found for hepatic fat (Table 5). On the basis of activity per total liver the mean value of the Casein I group was approximately one-third that of the Control group; the mean value of the Casein II group was about one-half that of the Control group. The mean value for the Casein I group per 100 gm of body weight was approximately one-half that of the Control group; for the Casein II group the mean value was about three-fifths that of the Control animals.

When mean values for FAO activities, for growth indices and for hepatic fat were examined in the Control, the Casein I and the Casein II groups, an increase in enzymic activity appeared to be associated with an increase in growth. The decrease in FAO activity observed in the casein-fed animals when compared with the control animals was accompanied by an increase in mean concentrations of hepatic fat. A linear relationship was obtained by plotting the logarithm of the uptake of oxygen per 10 minutes per 50 mg of liver or per total liver against the growth index values (Figure 2).

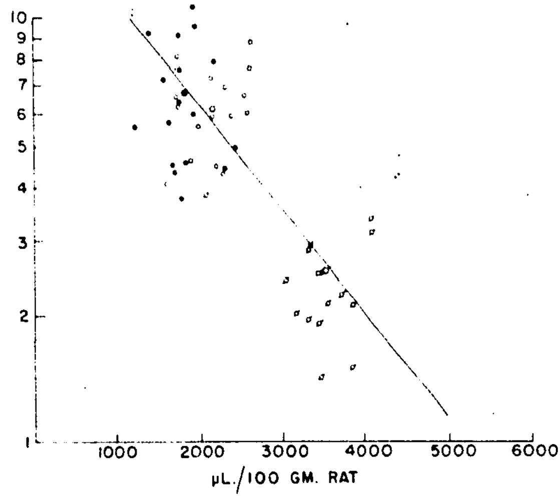
An increase in total activity with growth has been reported for some enzymes (Ross and Batt, 1957). Among the animals in the present experiment perhaps the highest values

would be expected in the control group since they had the highest mean growth index. Whether the increased activity of FAO in the control group can be attributed in part to a maturity or growth factor among these animals is not known. Experiments in which the activity of the FAO system is measured in the livers of rats of different sizes but maintained on the same ration are needed.

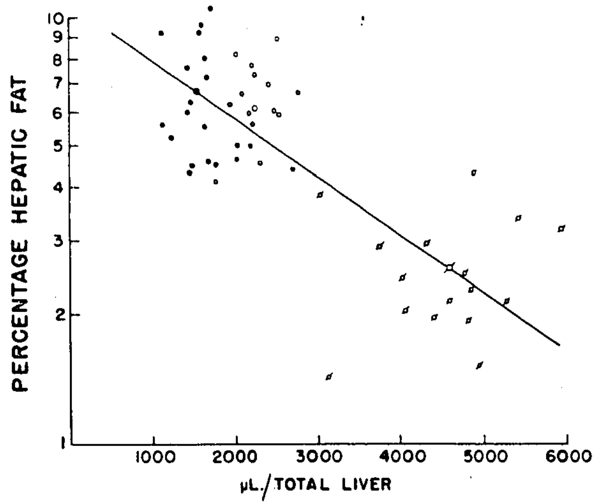
It has been assumed that the FAO system functions in the control of lipid accumulation in the liver. The highest mean FAO activity was found in the Control animals and was associated with the lowest mean hepatic fat observed among the 5 groups. An approximately linear relationship was obtained when the concentration of hepatic fat was plotted against the FAO activity per 100 gm of body weight (Figure 3). From this line an increase in hepatic fat of approximately 1% may be predicted for each 200 microliter decrease in the uptake of oxygen by the FAO system.

The significantly higher rate of enzymic activity observed when the Casein II was compared with the Casein I group may indicate the effect of an improved amino acid pattern of the dietary protein. The increased rate of FAO activity was associated with a decrease in the accumulation of hepatic lipids in the Casein II group as compared with mean values for the Casein I animals, although this difference in hepatic fat was not statistically significant at $P = 0.05$ (Duncan, 1955).

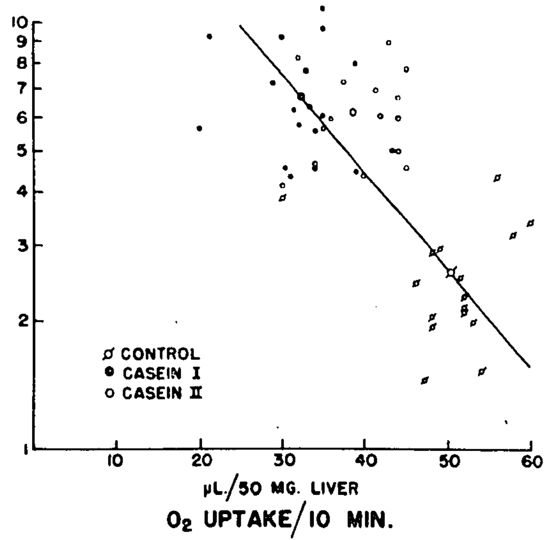
Figure 3. Relationship of hepatic fat concentration to FAO activity in animals fed Control, Casein I and Casein II diets



10
9
8
7
6
5
4
3
2
1
1000
2000
3000
4000
5000
6000
µL./100 GM. RAT



PERCENTAGE HEPATIC FAT
10
9
8
7
6
5
4
3
2
1
1000
2000
3000
4000
5000
6000
µL./TOTAL LIVER



10
9
8
7
6
5
4
3
2
1
CONTROL
CASEIN I
CASEIN II
10
20
30
40
50
60
µL./50 MG. LIVER
O2 UPTAKE/10 MIN.

When rates of FAO activity were compared for animals from the Wheat Gluten I and the Wheat Gluten II groups, a gain in weight and an increase in FAO activity were found in the group that received the lysine supplementation. However, in the Wheat Gluten II group the increase in FAO activity as measured in vitro was not accompanied by a decrease in hepatic fat. In vivo measurements would be desirable to determine whether the dietary limitations imposed by this ration prevented the FAO system from achieving its potential activity.

If the median hepatic fat value for the animals on the Wheat Gluten II ration were used to replace the mean value, the hepatic fat level in this group lay between the means for the Casein I and the Casein II groups but differences among the three were small (Table 5). Examination of the FAO activities in the Casein I, Casein II and Wheat Gluten II groups indicated that the mean FAO activity per 50 mg of liver in the Wheat Gluten II animals also lay between the mean activities of the two casein groups.

There is only a slight difference in mean FAO activity per total liver between the Wheat Gluten II and the Casein I groups. When the mean activity of the total liver of the Control group was assumed to be 100%, the mean activities per total liver of the Casein I and the Wheat Gluten II groups were calculated to be one-third of that found in the Control group. The difference between the mean FAO activity of the

Casein II group and that of the other three experimental groups was statistically significant at P = 0.05 (Duncan, 1955). Hence, measurements *in vitro* indicated that the Casein II group had the greatest potential FAO activity of all the experimental groups. However, the similarity among the hepatic fat concentrations in the Casein I, Casein II and Wheat Gluten II groups suggested that the markedly higher FAO activity observed among the Casein II animals was unable to function to capacity *in vivo* in the control of hepatic fat.

In comparisons of the mean FAO activity per 100 gm of body weight the order of relationship was altered and the activity of the Wheat Gluten II group was significantly greater than that of the Casein I group. Although the Wheat Gluten II also exceeded the Casein II group on the same basis, the difference was not significant.

Among the 4 groups maintained on the low protein rations in the present experiment, the highest mean potential FAO activities were not associated with the lowest concentrations of hepatic fat among individual animals or among groups of animals. Since there were no significant differences in the mean concentrations of hepatic fat among the Casein I, Casein II and Wheat Gluten I groups, it was not possible to establish meaningful relationships between FAO activity and hepatic fat from the data obtained in the experimental groups. It would be desirable to investigate possible changes in the transport

of hepatic lipids as a result of low protein diets in an effort to clarify fully differences in hepatic fat between control and experimental groups. A wider range in concentrations of fat in the livers of experimental animals should also aid in clarification of relationships between FAO activity and concentrations of hepatic fat. An increase in the proportion of the most-limiting amino acid in a low protein diet might increase the range in hepatic fat among experimental animals.

The animals from the Casein I group did not manifest a statistically significant superiority over animals on the Wheat Gluten I ration in the maintenance of FAO activity per 100 gm of body weight. However, mean FAO activities among animals receiving the Casein II and Wheat Gluten II diets were statistically higher than those observed on either unsupplemented ration. There was no significant difference between activities of the Casein II and Wheat Gluten II groups.

Endogenous Uptake of Oxygen in FAO Determinations

The volume of oxygen uptake per 50 mg of liver at 10 minutes in the absence of added octanoic acid was inversely related to the rate of FAO activity among the 5 groups of animals (Table 7). The Wheat Gluten I group with the lowest mean FAO activity per unit of liver had a mean endogenous uptake of oxygen approximately 1.5 times that observed in the

Table 7. Mean endogenous oxygen uptake by homogenates containing 50 gm of liver, mean hepatic fat per 50 mg of liver and FAO activity per 10 minutes per 50 mg of liver

Group	Number of animals	Mean endogenous oxygen uptake (microliters)	(%)	Hepatic fat per 50 mg liver (mg)	Mean FAO activity per 50 mg liver (microliters)
Con.	16	35.9 ± 9.4[a]	100	1.28	50.3 ± 6.7
Cas. I	17	44.9 ± 4.6	125	3.35	32.7 ± 5.8
Cas. II	17	42.2 ± 4.4	117	3.08	38.7 ± 5.3
W.G. I	17	54.5 ± 6.7	152	2.88	29.6 ± 3.5
W.G. II	17	43.1 ± 3.9	120	4.19	36.4 ± 9.6

[a]Standard deviation.

Control group.

It is not known whether or not the increased endogenous activity can be attributed to the higher percentage of hepatic fat found in animals on the purified diets as compared with those from the Control group. Waterlow (1952) raised the question as to whether endogenous uptake of oxygen in animals with hepatic fat concentrations exceeding those on a stock ration could be attributed to the respiration of hepatic fat. The addition of 0.3 ml of a solution of octanoic acid to each Warburg flask for the determination of FAO activity represented the addition of 0.575 mg of the fatty acid. The mean content of fat per 50 mg of liver from the Control group was 1.28 mg or about 2.2 times the amount of fat added as substrate for a single determination. The same weight of liver from the Wheat Gluten II group provided 4.19 mg of hepatic fat or approximately 7.2 times the amount of added substrate. The mean content of fat for the Wheat Gluten I group was 2.88 mg which lay between the two extremes represented by the Control and Wheat Gluten II groups (Table 7). However, the endogenous uptake of oxygen for the Wheat Gluten I group exceeded that of all other groups. In this experiment the rate of endogenous uptake of oxygen was not increased with increases in hepatic fat among either individual animals within groups or among groups of animals on purified diets. On the other hand, the mean endogenous uptake of oxygen per 50 mg of liver

among the Casein I, Casein II and the Wheat Gluten II groups were similar. Concentrations of hepatic fat among these three groups also did not differ significantly.

Srinivasan and Patwardhan (1955) found considerably lowered oxygen consumption in the livers of growing rats following 4 weeks on a protein-depletion diet. In the present experiment the animals in the Wheat Gluten I group were sufficiently restricted to show a mean growth index of 0.98. However, there was no apparent reduction in the mean endogenous oxygen uptake for the group. The mean endogenous oxygen uptakes for the Casein I, Casein II and Wheat Gluten II groups were less than that of the Wheat Gluten I animals although each of the mean growth indices for the three groups exceeded that of the Wheat Gluten I group.

Mean endogenous oxygen uptakes for the 4 experimental groups were greater than that of the Control animals, hence the responses of these animals to restricted dietary intakes did not duplicate the effects of protein-depletion reported by Srinivasan and Patwardhan (1955).

Activity of the G-6-P and 6-P-G Dehydrogenases

Changes in optical density due to formation of TPNH from TPN were obtained as a result of the reaction representing the summation of the activities of the G-6-P and 6-P-G dehydrogenases. From plotted data the rate of activity per

5 mg of liver, per total liver and per 100 gm of body weight were computed. The mean values for the control and for the experimental groups together with the corresponding standard deviations are given in Table 8.

In contrast to findings for the FAO system the mean of the summation of activities for the dehydrogenases per unit of liver or per unit of body weight was lower in animals from the control group than among those maintained on the purified rations. However, when the activities were calculated on the basis of the total liver the greatest enzymic activity was again found in the control group.

When mean activities per unit of liver or per unit of body weight were compared for the Control, Casein I and Casein II groups the highest activity was found in the Casein I group. The mean activity per 5 mg of liver among the Casein I animals was 146% of that in the Control group. The mean values per 5 mg of liver and per 100 gm of body weight for the Casein II group were between those for the Control and Casein I groups.

Comparisons of growth indices and the activities of the dehydrogenases for the Control, Casein II and Casein I groups indicated that a decrease in the activity of the dehydrogenases was present with an increase in the rate of growth. On the other hand, in these same groups an increase in hepatic fat was accompanied by an increase in the activity of these

Table 8. Mean activity of the G-6-P and 6-P-G dehydrogenases per 5 mg of liver, per total liver and per 100 gm body weight

Group	Number of animals	(Change in optical density in millimicrons per 4 minutes) Per 5 mg of liver (millimicrons)	(%)	Per total liver (millimicrons)	(%)	Per 100 gm body weight (millimicrons)	(%)
Con.	17	0.0250 ± 0.005[a]	100	23.3 ± 6.0	100	17.6 ± 4.0	100
Cas. I	17	0.0462 ± 0.011	185	21.8 ± 5.9	93	25.7 ± 6.2	146
Cas. II	17	0.0337 ± 0.006	135	19.8 ± 4.1	83	18.9 ± 4.3	108
W.G. I	17	0.0348 ± 0.008	139	11.8 ± 3.3	50	20.4 ± 5.3	116
W.G. II	17	0.0338 ± 0.010	135	14.3 ± 3.8	61	20.6 ± 5.8	117

[a]Standard deviation.

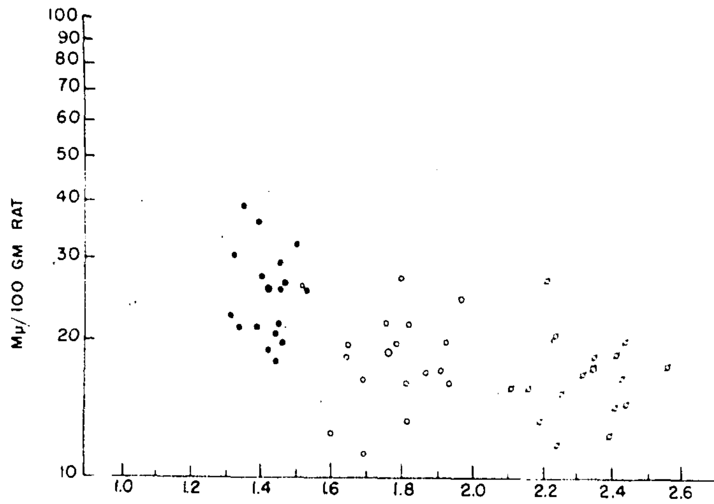
enzymes.

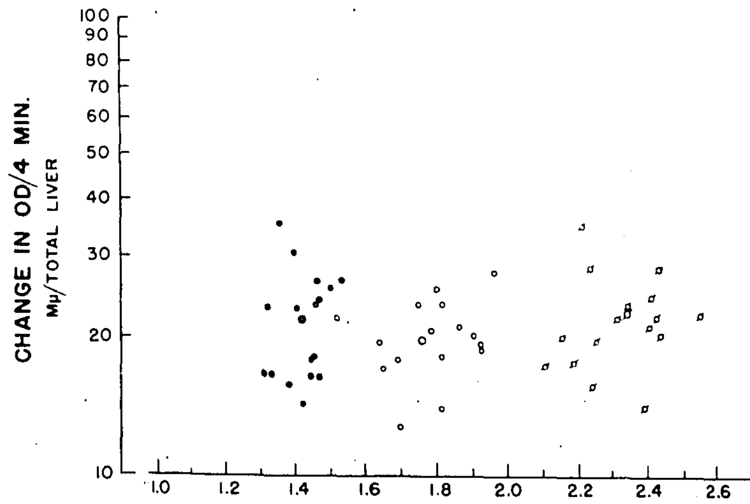
Plotting data for activity expressed per unit of liver or per unit of body weight against the growth index indicated a trend toward decreases in enzymic activity with accelerations in growth but the relationship was not linear. Increased amounts of hepatic fat were observed with increases in the activity of the dehydrogenases per unit of body weight or per unit of liver but again, the relationship was not linear. The use of semi-logarithm plots (Figures 4 and 5) did not clarify the relationship of the activity of the dehydrogenases to hepatic fat, but increases in growth index appeared to be related linearly to decreases in dehydrogenase activity per 5 mg of liver, although this relationship was not established for the other expressions of enzymic activity.

Mean values for the activity of the dehydrogenases per unit of liver were similar for animals in the Wheat Gluten I and Wheat Gluten II groups. When expressed as a percentage of the activity found in the Control group these activities were 139 and 135% for Wheat Gluten I and Wheat Gluten II animals respectively, hence no statistically significant difference was noted as the result of the lysine supplement. Among the animals in this experiment higher activities of the dehydrogenases per unit of liver were found in suboptimal protein states than in the Control group.

The activities of the dehydrogenases per unit of body

Figure 4. Relationship of activity of G-6-P and 6-P-G dehydrogenases to growth index in animals fed Control, Casein I and Casein II diets


Mμ/100 GM RAT
100
90
80
70
60
50
40
30
20
10
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6


CHANGE IN OD/4 MIN.
Mμ/TOTAL LIVER
100
90
80
70
60
50
40
30
20
10
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6

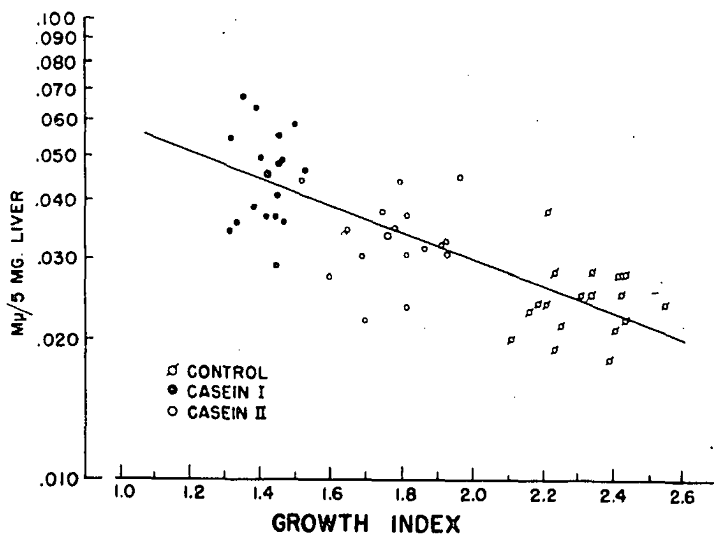
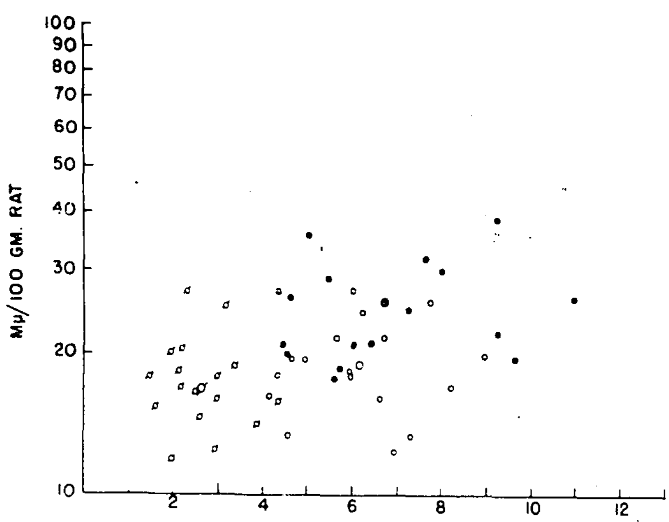
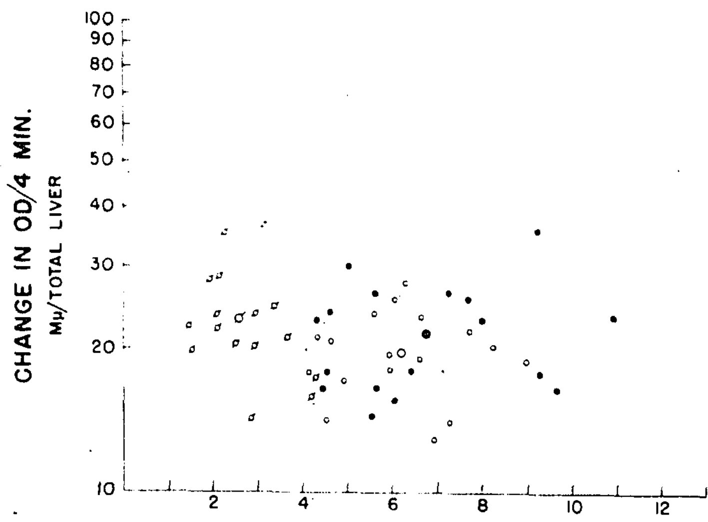
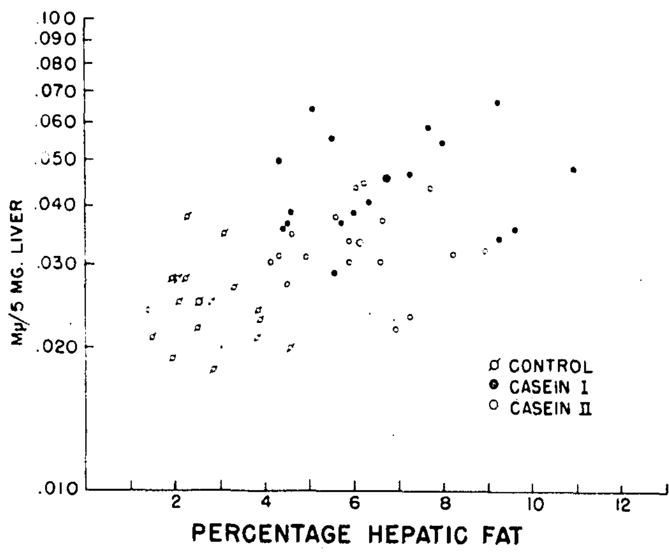

Mμ/5 MG. LIVER
.100
.090
.080
.070
.060
.050
.040
.030
.020
.010
CONTROL
CASEIN I
CASEIN II
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
GROWTH INDEX

Figure 5. Relationship of activity of G-6-P and 6-P-G dehydrogenases to hepatic fat concentration in animals fed Control, Casein I and Casein II diets


Mμ/100 GM. RAT


CHANGE IN OD/4 MIN.
Mμ/TOTAL LIVER


Mμ/5 MG. LIVER
CONTROL
CASEIN I
CASEIN II
PERCENTAGE HEPATIC FAT

weight of the Wheat Gluten I and Wheat Gluten II animals exceeded those of the Casein II group but the differences among the three groups were not significant (Duncan, 1955). The mean activity of animals in the Casein I group was significantly higher per 100 gm of body weight than that found among any of the other groups including that of the Control.

The differences in the mean weights of the livers among the groups appeared sufficient to account for the differences in activity per total liver. The mean activity per total liver of the casein-fed groups was significantly greater than that of the wheat gluten groups. The rate of activity per total liver among the Casein I animals was higher than that of the Casein II animals but this difference was not significant. The Wheat Gluten II animals also had a higher rate of activity than that found among animals on the unsupplemented wheat gluten ration. However, this difference, too, was not statistically significant at $P = 0.05$ (Duncan, 1955).

The mean activity per 100 gm of body weight for the Wheat Gluten I and Wheat Gluten II groups was computed as 116 to 117% of the mean activity found in the Control group from determinations *in vitro*. Among these animals where growth retardations were imposed by inadequate dietary intakes during the experimental period, some catabolism of hepatic protein to compensate for the dietary nitrogen deficiency was expected. Although a higher growth index was obtained for animals fed

the casein diets than for those maintained on wheat gluten, only the mean enzymic activity per 100 gm of body weight of the Casein I group exceeded that of the two groups receiving wheat gluten.

Weber (1959) classified the HMP shunt dehydrogenases as enzymes which were catabolized in preference to cytoplasm under conditions where dietary protein was inadequate to meet body needs. Activities obtained among the Wheat Gluten I and Wheat Gluten II animals in this experiment were not diminished if the mean activity of the Control group were assumed to be 100%. Activities per unit of liver and per 100 gm of body weight among the casein-fed animals also exceeded those of the control animals although these animals were also receiving less than optimal amounts of protein for growth.

Results of enzyme assays among animals on the experimental diets versus those among Control animals were also examined in relationship to a theory of reduced enzymic activity due to a decrease in available substrate. Tepperman and Tepperman (1958) reported a depressed HMP shunt activity in conditions of altered carbohydrate metabolism observed in diabetes. The diabetic subject exhibited a decrease in the activity of the dehydrogenases which was explained as the result of an absence of sufficient substrate material to keep the shunt pathway in operation. It appears doubtful that the differences observed in the animals in this experiment may be

explained by the absence of substrate material since the rate of activity among the Wheat Gluten I animals with their severe growth retardation exceeded that found in the Control group. It would not be expected that the Wheat Gluten I animals could provide more glucose-6-phosphate than was present in the Control group.

The HMP shunt pathway appears to operate in order to provide a pool or reserve of reduced TPN and to produce ribose 5-phosphate essential for nucleic acid synthesis (Weber, 1959). It has been suggested that lipogenesis cannot proceed in the absence of reduced TPN. Langdon (1955) demonstrated the role of TPNH in the reduction of crotonyl-CoA to butyryl-CoA and suggested the possibility that TPNH was the specific electron donor required for the reduction of the β-unsaturated acyl-CoA derivatives. He further proposed that the depressed lipogenesis found in diabetes be attributed to the reduced availability of TPNH which resulted from the diminished activity of the HMP shunt pathway.

It would appear that a knowledge of the glycogen content of the liver after fasting for 24 hours might also be necessary to clarify differences in the mean activities of the dehydrogenases among these groups of animals. Although in preliminary experiments the use of fasting periods of 12, 15, 18 and 24 hours appeared to make little, if any, difference in the activities of the dehydrogenases in animals maintained on

the stock ration, it would be desirable to know whether a different response might follow similar variations in the fasting time of the Wheat Gluten I animals, for instance.

Wakil et al. (1959) concluded that TPNH was the sole electron donor for the synthesis of fatty acids in the avian liver. However, this specificity for electron donors did not hold for synthesis in all species or in all kinds of tissue.

When results of the present experiment were examined against a theory of the dependence of lipogenesis on the activity of the HMP shunt pathway, the activity per total liver, in vitro, should be adequate to supply the TPNH necessary for the synthesis of even more fat than was observed in the livers of the 4 experimental groups. Higher hepatic fat levels were found among animals on purified diets than among those on the stock diet and the dehydrogenase activities per unit of liver or per unit of body weight among these animals also exceeded the activity determined in the control group.

The findings of Lee and Lucia (1961) were of interest when the differences in magnitude of dehydrogenase activities were considered among the 5 groups of animals. Determinations in vivo of the activity of the HMP pathway and of the glycolytic pathway resulted in a ratio of 1.87 in mature animals when no caloric restrictions were imposed. A ratio of 0.9 was obtained when caloric restriction was imposed on animals maintained on the same diet. Approximately 50% more

activity in the HMP shunt pathway was observed by Lee and Lucia among animals on the non-restricted than among those on restricted diets when the same ration was used. However, when activity was determined in vitro using liver homogenates in a determination similar to that used in the present experiment, a marked increase in the G-6-P dehydrogenase and little difference in the 6-P-G dehydrogenase activity were observed with caloric restriction. On the basis of their results Lee and Lucia concluded that the decreased activity of the shunt as measured in vivo could not be attributed to a loss in potential activity of the enzymes.

If the Wheat Gluten I animals in this experiment were considered as an example of partial caloric restriction the determinations in vitro of dehydrogenase activities per unit of liver or per unit of body weight were higher than those of the Control animals. This finding suggests no loss of potential activity. It would be interesting to know whether the activities in vivo in these animals followed the pattern described by Lucia and Lee.

A theory proposed by Fitch and Chaikoff (1960) offered a possible explanation of apparent "excesses" of activity in experimental compared with control animals. He proposed that increased activity of enzymes might result from stoppage of breakdown of the enzymes themselves, resulting in a reduced turnover of enzymes. It is not known whether the dietary

conditions in this experiment created a block in the normal turnover of these enzymes.

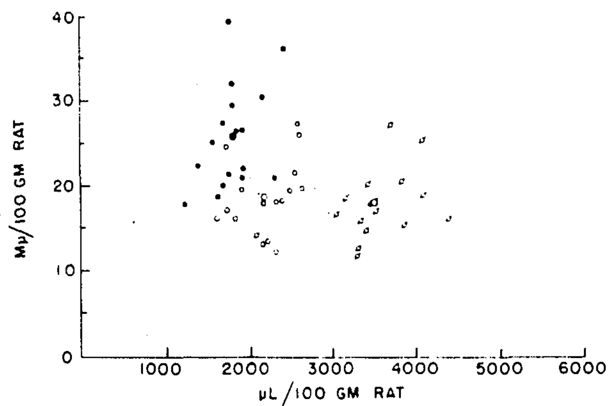
Relationships between the FAO System and the G-6-P and 6-P-G Dehydrogenases

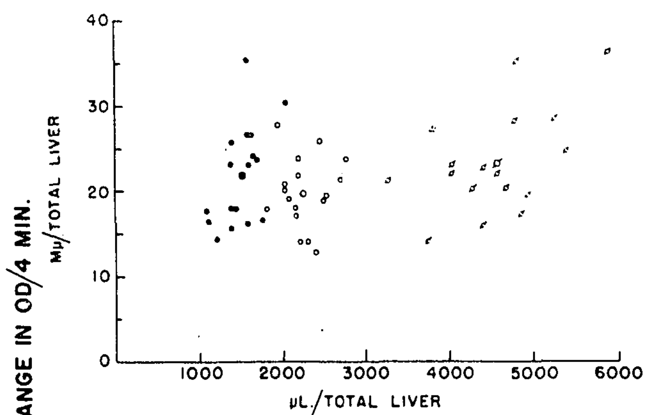
Increased activities of the FAO system appeared to accompany increased growth index values and were associated with decreased hepatic fat levels in the control and in the casein-fed animals. An inverse relationship of activities of the dehydrogenases to growth indices was observed among these three groups. Data from the groups were examined to determine whether a pattern of change in the activity of one enzyme system was present with a measured change in the activity of the second system. A decrease in the activity of one enzyme system was observed with an increased activity of the second among the animals in this experiment but the relationship was not linear (Figure 6).

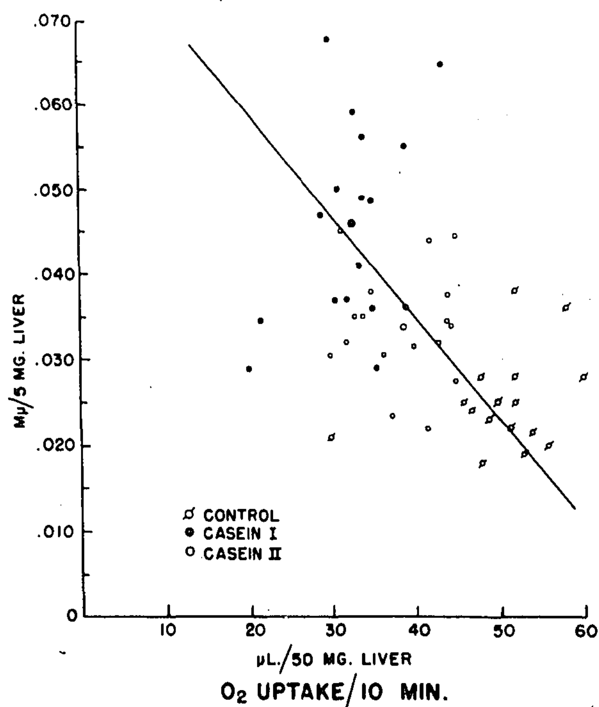
The possibility that the two enzyme systems are not of equal importance in function may explain the lack of linearity as well as some of the variations among individual animals and among groups. Four pathways are available for the metabolism of glucose-6-phosphate but it is doubtful that alternate pathways exist for fatty acid metabolism.

Brauer (1956) suggested the possibility of greater stability among enzyme systems associated with intracellular

Figure 6. Relationship of activity of G-6-P and 6-P-G dehydrogenases to FAO activity in animals fed Control, Casein I and Casein II diets


Mμ/100 GM RAT
μL/100 GM RAT
0
10
20
30
40
1000
2000
3000
4000
5000
6000


CHANGE IN OD/4 MIN.
Mμ/TOTAL LIVER
μL./TOTAL LIVER
0
10
20
30
40
1000
2000
3000
4000
5000
6000


Mμ/5 MG. LIVER
.070
.060
.050
.040
.030
.020
.010
0
10
20
30
40
50
60
CONTROL
CASEIN I
CASEIN II
μL./50 MG. LIVER
O_2 UPTAKE/10 MIN.

formed elements in comparison with those in solution in the cytoplasm. Hence, a greater sensitivity to dietary alterations might be expected in the dehydrogenases than in the FAO system.

In the experiment reported here, activities of the dehydrogenases per unit of liver increased among animals on purified diets in which the amounts of protein were suboptimal for growth when compared with activities found in animals on the stock ration. Comparisons on the same basis between animals on the stock ration and those maintained on purified diets indicated a decrease in FAO activity in response to a suboptimal amount of protein.

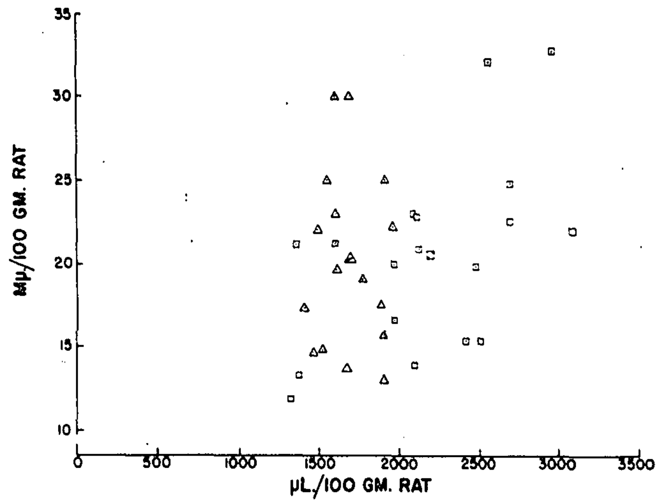
Ratios of total FAO activity to total HMP shunt activity were highest in the Control animals with a value of 197 followed in descending order by Casein II, 114; Wheat Gluten II, 107; Wheat Gluten I, 84 and Casein I, 70. The high ratio of FAO to HMP shunt activity which appeared in the control group was associated with the lowest concentration of hepatic fat found among the 5 groups.

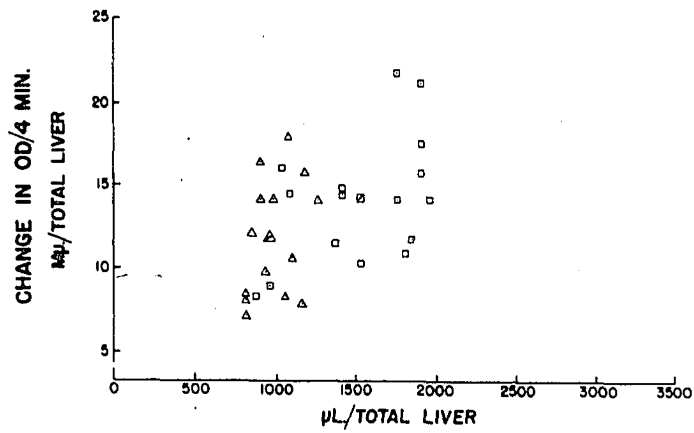
A greater variability in concentrations of hepatic fat would be desirable to provide further information to clarify any relationship between alterations in the HMP dehydrogenases, the FAO system and concentrations of hepatic fat.

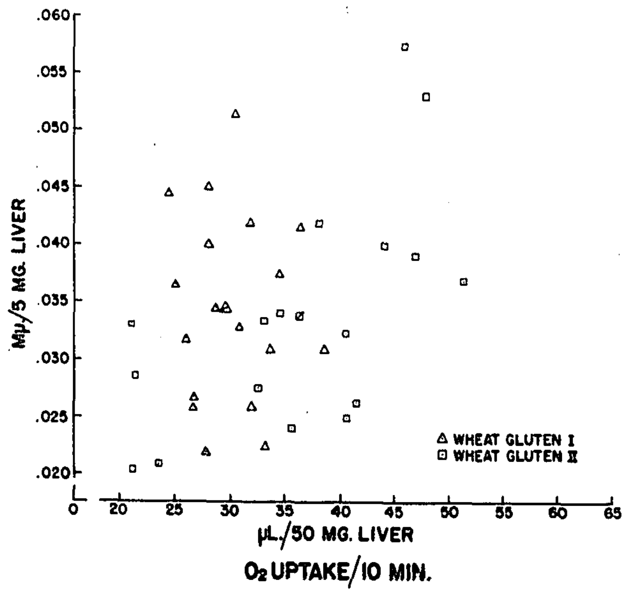
When the activity of one enzyme system was plotted against that of the other using only the values from animals in

the two wheat gluten groups, a slight increase in the rate of activity per total liver was observed in one enzyme system with an increase in the rate of the second. Plotting values per 100 gm rat or per unit of liver for the two systems suggested no trend at all (Figure 7).

Figure 7. Relationship of activity of G-6-P and 6-P-G dehydrogenases to FAO activity in animals fed Wheat Gluten I and Wheat Gluten II diets


MU/100 GM. RAT
µL./100 GM. RAT


CHANGE IN OD/4 MIN.
MU/TOTAL LIVER
µL./TOTAL LIVER


MU/5 MG. LIVER
WHEAT GLUTEN I
WHEAT GLUTEN II
µL./50 MG. LIVER
O2 UPTAKE/10 MIN.

SUMMARY AND CONCLUSIONS

Groups of 17 male weanling rats of the Wistar strain were maintained three weeks on purified diets in which either 9% casein or 9% wheat gluten was used as the sole dietary protein. Similar groups of animals received isonitrogenous diets containing 8.7% casein plus 0.3% methionine or 8.7% wheat gluten plus 0.3% lysine. A fifth group of animals was fed the laboratory stock ration and served as a control group.

Records of food consumption and weights were kept for individual animals. A growth index value was calculated for each animal by the formula:

$$\frac{\text{weight after 3 weeks on experimental diets}}{\text{weight at weaning}} .$$

All animals gained weight except those maintained on the unsupplemented wheat gluten ration. At the end of the experimental period the animals were fasted 24 hours and sacrificed by stunning and decapitation. The livers were assayed for fat content and the activities of FAO and the combined G-6-P and 6-P-G dehydrogenase system were determined and expressed per unit weight of liver, per total liver and per 100 gm of body weight.

The mean FAO activity of the control group exceeded that of all experimental groups. FAO activity increased with increases in the growth index and increases in mean FAO activity were associated with decreases in concentrations of hepatic

fat when the animals in the unsupplemented wheat gluten group were omitted from the comparison.

Similar activities of the FAO system per 50 mg of liver or per 100 gm of body weight were observed among animals fed the unsupplemented casein or unsupplemented wheat gluten rations. Mean FAO activities per unit of liver or per unit of body weight in the animals on the two supplemented protein rations were also similar and were significantly higher than those found in the unsupplemented groups. These findings suggested no superiority of casein over wheat gluten in the maintenance of potential FAO activity as determined _in vitro_, but a higher rate of activity was obtained when each protein was supplemented with 0.3% of its most-limiting amino acid.

The desirability of _in vivo_ determinations of enzymic activity and of further investigations of factors which function in the regulation of hepatic fat was indicated from the observation that, among animals receiving purified rations, the lowest hepatic fat and the lowest rate of FAO activity were present together in the unsupplemented wheat gluten group. However, this group of animals also showed a voluntary reduction in food intake and a growth index less than 1.0.

The endogenous respiration rates observed among these animals were different from those reported in the literature in protein deficiency. The lowest rate of endogenous respiration was associated with the maximum growth index and

maximum FAO activity in the Control group. The highest endogenous rate was found among animals in the Wheat Gluten I group in combination with the lowest FAO activity and the minimum growth index.

The highest mean activity per 5 mg of liver or per 100 gm of body weight for the HMP shunt dehydrogenases was found in the unsupplemented casein group, the lowest in the control group which also had the lowest mean hepatic fat among the 5 groups. The mean concentration of hepatic fat in the Casein I group was exceeded only by the mean value for the Wheat Gluten II group. When the median value was substituted for the mean concentration of hepatic fat in the Wheat Gluten II group, the highest hepatic fat as well as the highest activity per 5 mg of liver or per 100 gm of body weight for the dehydrogenases in the 5 groups were observed in the Casein I group.

However, when the mean activities per total liver for the dehydrogenases were compared, the control group exceeded the experimental groups. The highest mean total amount of fat per liver was 0.182 gm and was found in the Casein II group. Mean total hepatic fat per liver in the remaining groups in descending order of magnitude were Wheat Gluten II, Casein I, Control and Wheat Gluten I.

The maintenance of a high activity of the dehydrogenases on the unsupplemented casein ration in contrast to a com-

paratively low FAO activity when the same ration was used suggested the probability of unlike requirements of dietary protein for the two systems. Since an approximately linear relationship between FAO activity and concentrations of hepatic fat was found from data for the control and casein-fed animals and no similar relationship was apparent between the activities of the dehydrogenases and the concentration of hepatic fat, it would appear that the FAO system may be more directly related to the regulation of hepatic fat than are the dehydrogenases. The concentration of hepatic fat and the FAO activity per total liver found in the control and experimental groups also suggested the importance of the FAO system in the control of hepatic fat.

Comparisons using the control and casein-fed animals also suggested a decrease in the activity of one enzymic system with an increase in the activity of the second although no linear relationship was found.

The highest ratio of FAO to HMP shunt dehydrogenase activity in the 5 groups was found in the control group in which the lowest mean hepatic fat concentration also was observed.

Differences in mean activities of FAO and HMP shunt dehydrogenases among groups may be influenced by growth, the former increasing, the latter decreasing with accelerations in growth.

Failure to produce large differences in concentrations of hepatic fat among the 4 experimental groups made it impossible to investigate relationships between the function of enzymes as determined *in vitro* and hepatic fat except between the control and the experimental groups. Additional experiments are needed in which greater differences in hepatic fat are produced. The addition of larger proportions of methionine to the 9% casein ration and of as much as 0.45% lysine to the 9% wheat gluten ration might be used to feed additional groups. The use of a control group maintained on a synthetic diet containing 18% casein might permit a more direct determination of the effect of low protein rations on activities of hepatic enzymes.

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APPENDIX

Table 9. Individual data for animals in the control group

Rat number	Weaning weight (gm)	Autopsy weight (gm)	Growth index	Average daily food intake (gm)	Liver weight (gm)	Hepatic fat (%)
I-A- 1	68	148	2.41	10.8	5.05	3.86
- 2	60	111	2.11	8.7	4.36	4.30
- 3	67	134	2.24	9.5	4.20	1.97
- 4	70	138	2.24	9.9	5.09	2.14
- 5	70	133	2.19	9.8	5.08	2.96
I-B- 6	55	114	2.40	8.2	3.92	2.88
- 7	55	128	2.56	8.8	4.72	1.44
- 8	62	134	2.43	9.2	4.42	2.42
- 9	66	129	2.26	8.9	4.60	1.52
-10	62	129	2.35	9.2	4.24	2.04
-11	60	145	2.75	9.7	5.10	3.17
I-C-12	62	133	2.42	9.7	4.52	3.38
-13	63	130	2.32	9.3	4.40	2.14
-14	64	139	2.44	8.6	4.62	2.51
-15	64	140	2.44	9.4	5.01	1.93
-16	67	129	2.16	8.6	4.40	2.94
-17	66	130	2.22	8.7	4.63	2.28

Table 10. Individual data for animals in the control group

Rat number	FAO activity			Endogenous oxygen uptake	Activity of the G-6-P and 6-P-G dehydrogenases		
	Uptake of oxygen in microliters per 10 minutes				Change in optical density in millimicrons per 4 minutes		
	50 mg liver	total liver	100 gm body weight	50 mg liver	5 mg liver	total liver	100 gm body weight
I-A- 1	30.0	3030	2047	26.4	0.0210	21.21	14.30
- 2	56.0	4883	4399	39.1	.0200	17.44	15.70
- 3	53.0	4431	3306	30.7	.0190	15.88	11.90
- 4	52.0	5294	3836	36.2	.0280	28.50	20.60
- 5	--	--	--	--	.0240	23.84	17.90
I-B- 6	48.0	3763	3301	35.9	.0180	14.11	12.40
- 7	47.0	4437	3466	39.2	.0240	22.66	17.70
- 8	46.0	4066	3035	32.8	.0250	22.10	16.50
- 9	54.0	4968	3851	35.8	.0215	19.78	15.30
-10	48.0	4070	3155	40.2	.0280	23.74	18.40
-11	58.0	5939	4096	35.8	.0355	36.35	25.10
I-C-12	60.0	5424	4078	35.8	.0275	24.86	18.70
-13	52.0	4576	3520	39.3	.0250	22.00	16.90
-14	51.5	4759	3423	37.8	.0220	20.33	14.60
-15	48.0	4810	3435	37.0	.0280	28.06	20.00
-16	49.0	4312	3343	37.0	.0230	20.24	15.70
-17	52.0	4815	3704	35.9	.0380	35.19	27.10

Table 11. Individual data for animals maintained on unsupplemented casein diet

Rat number	Weaning weight (gm)	Autopsy weight (gm)	Growth index	Average daily food intake (gm)	Liver weight (gm)	Hepatic fat (%)
II-A- 1	64	79	1.31	6.1	2.57	9.27
- 2	60	77	1.42	6.2	1.95	5.74
- 3	68	89	1.44	7.0	2.43	4.56
- 4	65	84	1.40	6.3	2.30	4.36
- 5	70	93	1.44	7.1	2.84	5.61
II-B- 6	58	74	1.38	5.2	2.00	6.03
- 7	68	91	1.47	6.6	2.46	4.60
- 8	63	78	1.33	6.0	2.30	4.47
- 9	62	84	1.45	6.1	2.19	6.38
-10	63	76	1.32	5.8	2.10	8.00
-11	66	84	1.39	6.2	2.35	5.06
II-C-12	58	80	1.50	7.2	2.17	7.68
-13	65	90	1.46	7.3	2.38	5.56
-14	71	90	1.35	7.1	2.62	9.21
-15	61	83	1.47	6.4	2.28	9.63
-16	74	105	1.53	6.6	2.83	7.27
-17	65	89	1.46	7.5	2.43	10.94

Table 12. Individual data for animals maintained on unsupplemented casein diet

Rat number	FAO activity			Endogenous oxygen uptake	Activity of the G-6-P and 6-P-G dehydrogenases		
	Uptake of oxygen in microliters per 10 minutes				Change in optical density in millimicrons per 4 minutes		
	50 mg liver	total liver	100 gm body weight	50 mg liver	5 mg liver	total liver	100 gm body weight
II-A -1	21.5	1105	1398	36.1	0.0345	17.73	22.40
-2	32.0	1248	1621	49.7	.0370	14.43	18.70
-3	30.5	1482	1666	40.0	.0370	17.98	20.20
-4	31.0	1426	1698	44.1	.0500	23.00	27.40
-5	20.0	1136	1222	41.8	.0290	16.47	17.70
II-B -6	35.5	1420	1919	36.8	.0390	15.60	21.10
-7	34.0	1673	1838	43.2	.0490	24.11	26.50
-8	39.0	1794	2300	46.4	.0360	16.56	21.20
-9	33.5	1468	1747	45.0	.0410	17.96	21.40
-10	39.0	1638	2155	43.7	.0550	23.10	30.40
-11	43.5	2044	2434	44.7	.0645	30.32	36.10
II-C-12	33.0	1432	1790	49.7	.0590	25.61	32.00
-13	34.0	1618	1798	48.1	.0560	26.66	29.60
-14	30.0	1572	1747	53.2	.0675	35.37	39.30
-15	35.0	1596	1923	44.1	.0360	16.42	19.80
-16	29.0	1641	1563	48.6	.0470	26.60	25.30
-17	35.0	1701	1911	48.1	.0485	23.57	26.50

Table 13. Individual data for animals maintained on diet with casein plus methionine

Rat number	Weaning weight (gm)	Autopsy weight (gm)	Growth index	Average daily food intake (gm)	Liver weight (gm)	Hepatic fat (%)
III-A- 1	55	96	1.93	7.5	2.92	8.94
- 2	67	104	1.70	7.3	2.90	6.98
- 3	71	105	1.60	5.9	2.56	4.54
- 4	68	118	1.87	8.2	3.38	4.35
- 5	62	101	1.82	7.2	2.97	5.98
III-B- 6	63	105	1.82	7.4	2.98	7.29
- 7	65	107	1.78	7.3	2.98	4.61
- 8	57	88	1.65	6.3	2.47	4.97
- 9	56	95	1.80	7.2	2.93	6.02
-10	64	114	1.97	8.6	3.10	6.26
-11	66	110	1.82	8.0	3.16	6.65
III-C-12	70	107	1.64	7.8	2.87	5.94
-13	71	111	1.69	8.1	2.94	4.13
-14	68	119	1.91	8.4	3.17	8.23
-15	68	111	1.76	8.2	3.15	5.61
-16	68	120	1.93	8.9	3.15	6.60
-17	59	85	1.52	6.6	2.46	7.74

Table 14. Individual data for animals maintained on diet with casein plus methionine

	FAO activity			Endogenous oxygen uptake	Activity of the G-6-P and 6-P-G dehydrogenases		
	Uptake of oxygen in microliters per 10 minutes				Change in optical density in millimicrons per 4 minutes		
Rat number	50 mg liver	total liver	100 gm body weight	50 mg liver	5 mg liver	total liver	100 gm body weight
III-A- 1	43.0	2511	2616	43.6	0.0325	18.98	19.80
- 2	41.5	2407	2314	35.9	.0220	12.76	12.30
- 3	45.0	2304	2194	43.7	.0275	14.08	13.40
- 4	40.0	2704	2292	38.9	.0315	21.29	18.00
- 5	36.5	2168	2146	45.2	.0305	18.11	17.90
III-B- 6	37.5	2235	2128	38.7	.0235	14.00	13.30
- 7	34.0	2026	1893	39.6	.0350	20.86	19.50
- 8	44.0	2174	2470	36.1	.0345	17.04	19.40
- 9	42.0	2461	2590	39.9	.0440	25.78	27.10
-10	31.5	1953	1713	47.7	.0450	27.90	24.50
-11	44.0	2781	2528	46.2	.0375	23.70	21.50
III-C-12	44.5	2554	2387	35.6	.0340	19.52	18.20
-13	30.0	1764	1589	40.9	.0305	17.93	16.20
-14	32.0	2029	1705	49.9	.0320	20.29	17.00
-15	35.0	2205	1986	47.0	.0380	23.94	21.60
-16	33.0	2079	1732	45.6	.0305	19.22	16.00
-17	45.0	2214	2605	42.1	.0445	21.89	25.80

Table 15. Individual data for animals maintained on unsupplemented wheat gluten diet

Rat number	Weaning weight (gm)	Autopsy weight (gm)	Growth index	Average daily food intake (gm)	Liver weight (gm)	Hepatic fat (%)
IV-A- 1	64	60	1.02	5.0	1.74	8.14
- 2	58	52	.96	4.8	1.59	7.50
- 3	55	53	1.05	5.4	1.60	5.34
- 4	68	62	.97	4.6	1.87	4.92
- 5	68	60	.94	4.9	1.84	3.32
IV-B- 6	60	54	.97	4.1	1.55	6.15
- 7	65	59	.98	4.4	1.67	4.02
- 8	60	55	1.00	4.2	1.67	6.91
- 9	61	56	.98	4.6	1.61	5.86
-10	68	60	.96	4.9	1.76	6.33
-11	61	55	.97	4.4	1.84	5.74
IV-C-12	59	52	.97	4.3	1.50	5.70
-13	64	57	.97	4.9	1.59	6.90
-14	68	61	.94	5.0	1.77	4.13
-15	70	64	.98	5.6	1.74	6.65
-16	65	57	.95	3.6	1.57	5.48
-17	66	60	.97	4.9	1.61	5.00

Table 16. Individual data for animals maintained on unsupplemented wheat gluten diet

Rat number	FAO activity: Uptake of oxygen in microliters per 10 minutes — 50 mg liver	total liver	100 gm body weight	Endogenous oxygen uptake — 50 mg liver	Activity of the G-6-P and 6-P-G dehydrogenases: Change in optical density in millimicrons per 4 minutes — 5 mg liver	total liver	100 gm body weight
IV-A- 1	33.0	1148	1913	36.5	0.0225	7.83	13.00
- 2	27.5	874	1682	48.9	.0220	7.00	13.50
- 3	32.0	1024	1932	44.8	.0260	8.32	15.70
- 4	32.0	1197	1930	57.6	.0420	15.71	25.30
- 5	34.0	1251	2085	50.8	.0375	13.80	23.00
IV-B- 6	26.5	822	1521	60.9	.0260	8.06	14.90
- 7	33.5	1119	1896	55.6	.0310	10.35	17.50
- 8	25.0	835	1518	63.2	.0365	12.19	22.20
- 9	28.5	918	1639	55.0	.0345	11.11	19.80
-10	30.5	1074	1787	54.0	.0515	18.13	30.20
-11	24.5	902	1639	56.8	.0445	16.38	29.80
IV-C-12	31.0	930	1788	53.1	.0330	9.90	19.00
-13	28.0	890	1562	51.3	.0450	14.31	25.10
-14	28.0	991	1625	62.7	.0400	14.16	23.20
-15	26.0	905	1414	58.9	.0320	11.14	17.40
-16	26.5	832	1460	60.7	.0265	8.32	14.60
-17	36.5	1175	1959	55.7	.0415	13.36	22.30

Table 17. Individual data for animals maintained on diet with wheat gluten plus lysine

Rat number	Weaning weight (gm)	Autopsy weight (gm)	Growth index	Average daily food intake (gm)	Liver weight (gm)	Hepatic fat (%)
V-A- 1	65	67	1.14	5.0	2.53	29.10
- 2	67	72	1.15	5.7	2.14	6.52
- 3	70	71	1.08	5.5	2.20	4.30
- 4	72	74	1.10	6.0	2.11	7.87
- 5	60	63	1.12	5.7	2.06	6.66
V-B- 6	62	67	1.18	5.4	2.08	12.69
- 7	67	71	1.15	5.3	2.20	4.40
- 8	64	70	1.17	5.8	2.04	4.70
- 9	61	65	1.15	5.7	2.00	3.52
-10	60	63	1.15	5.3	1.91	3.95
-11	62	71	1.24	5.6	2.16	6.36
V-C-12	64	75	1.26	6.9	2.20	4.54
-13	63	71	1.24	5.5	1.97	6.47
-14	67	68	1.07	5.1	1.91	4.84
-15	64	73	1.22	6.0	2.18	5.24
-16	61	68	1.18	5.4	2.09	12.72
-17	67	75	1.18	6.1	2.44	18.83

Table 18. Individual data for animals maintained on diet with wheat gluten plus lysine

	FAO activity			Endogenous oxygen uptake	Activity of the G-6-P and 6-P-G dehydrogenases		
	Uptake of oxygen in microliters per 10 minutes				Change in optical density in millimicrons per 4 minutes		
Rat number	50 mg liver	total liver	100 gm body weight	50 mg liver	5 mg liver	total liver	100 gm body weight
V-A- 1	21.50	1088	1623	37.7	.0285	14.42	21.50
- 2	35.50	1519	2110	39.3	.0240	10.27	14.30
- 3	40.50	1782	2510	40.5	.0250	11.00	15.50
- 4	23.50	992	1340	48.5	.0210	8.86	12.00
- 5	21.00	865	1373	40.2	.0205	8.45	13.40
V-B- 6	34.50	1435	2142	42.9	.0340	14.14	21.10
- 7	44.00	1936	2726	36.9	.0400	17.60	24.80
- 8	47.00	1918	2740	42.5	.0390	15.91	22.70
- 9	48.00	1920	2954	43.7	.0535	21.40	32.90
-10	51.50	1957	3106	44.8	.0370	14.06	22.30
-11	40.50	1750	2465	40.9	.0325	14.04	19.80
V-C-12	41.50	1826	2434	42.7	.0265	11.66	15.50
-13	38.00	1497	2108	49.3	.0420	16.55	23.30
-14	46.00	1757	2584	41.4	.0575	21.96	32.30
-15	33.00	1439	1971	45.1	.0335	14.61	20.00
-16	32.50	1358	1997	50.9	.0275	11.50	16.90
-17	21.00	1025	1367	45.2	.0330	16.10	21.50

Table 19. Composition of laboratory stock diet in grams per 1000 grams

Dietary components	Grams per 1000 gm of diet
Cornmeal	483
Linseed meal	138
Klim	138
Wheat germ	86
Yeast (unirradiated)	82
Casein	43
Alfalfa meal	17
Sodium chloride	4
$CaCO_3$ plus trace elements	4
Yeast (irradiated)	4

Abbreviations Used in Thesis

ATP	adenosine triphosphate
CoA	coenzyme A
FAO	fatty acid oxidase
G-6-P	glucose-6-phosphate
6-P-G	6-phosphogluconate
HMP	hexose monophosphate
TPN	triphosphopyridine nucleotide
TPNH	reduced triphosphopyridine nucleotide