EFFECT OF GLYCEROL ON LIPOGENIC ENZYME ACTIVITIES AND ON FATTY ACID SYNTHESIS IN THE RAT AND CHICKEN

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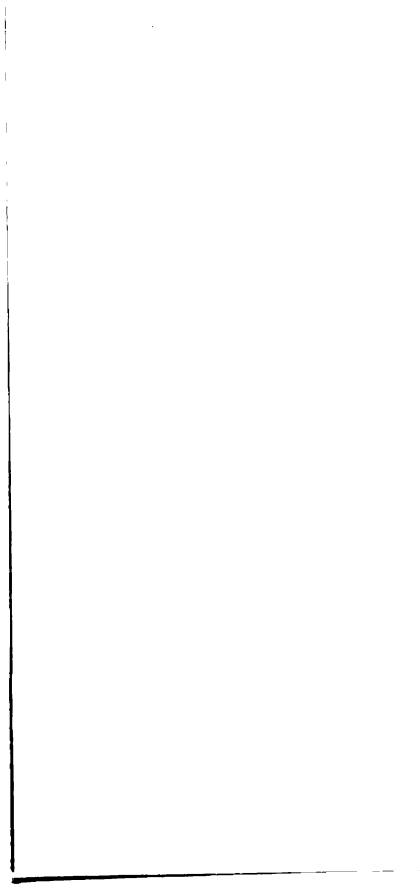

ABSTRACT

EFFECT OF GLYCEROL ON LIPOGENIC ENZYME ACTIVITIES AND ON FATTY ACID SYNTHESIS IN THE RAT AND CHICKEN

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The influence of glycerol addition to an in vitro system and of dietary glycerol on the lipid metabolism in the rat and chicken was studied. Buffer containing 3 or 10 mM glycerol depressed the rates of fatty acid synthesis in rat liver slices. However, buffer containing up to 25 mM glycerol did not influence the rates of fatty acid synthesis in chick liver slices. The in vitro inhibitory action of glycerol in rat liver slices might occur at the level of glycogen or glucose conversion to pyruvate.

Rats and chickens were fed a high-glucose diet or glycerol-containing diet for either 3 days or 3 weeks. Feeding diets containing 20-parts glycerol to rats or chickens did not influence the growth rate of the animals. However, substitution of 40-parts glycerol for glucose in the diet significantly depressed growth rate in both rats and chickens.

The liver tissue supernatants were assayed for citrate cleavage enzyme, fatty acid synthetase and malic enzyme activities. The in vivo rate of fatty acid synthesis was also determined. The activities of citrate cleavage enzyme, fatty acid synthetase and malic enzyme in livers of rats fed the glycerol-containing diets were dramatically increased. However, this stimulation of enzyme activity occurred without a concomitant increase in the in vivo rate of fatty acid synthesis in the rat liver. In the chicken, unlike the rat, dietary glycerol did not increase hepatic lipogenic enzyme activities.

No significant differences in adipose lipogenic enzyme activities and in the rate of fatty acid synthesis in rats fed glycerol-containing diets for 3 days or for 3 weeks were observed.

Apparently, the lipogenic response to glycerol feeding depends on the species as well as the organ examined.

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By

Ming Huey Lin

A THESIS

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DEDICATION

To my father and my mother

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I wish to express my sincere thanks to my academic advisor, Dr. Dale R. Romsos, for his guidance, encouragement and inspiration throughout this study. Thanks are also due to Dr. Maurice R. Bennink and Dr. William W. Wells, members of my graduate guidance committee, for their helpful discussions and suggestions.

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INTRODUCTION

It has been established that changes in the metabolism of protein, carbohydrate, and lipid during the development of the animal are associated with concomitant dietary changes. The composition of the diet influences the enzyme pattern found in various tissues (1, 2, 3, 4, 5, 6). Most studies on the metabolic regulation of lipogenesis have been conducted in the rat. Few studies have been conducted with other species. There are significant differences in the regulation of metabolism among various species (4, 7, 8, 9, 10). Detailed studies with a variety of animal models should provide basic information on the metabolic regulation of lipid metabolism useful to humans.

We have utilized the rat and the chicken as comparative animal models to study lipid metabolism, each has certain advantages and may resemble the human in certain aspects (11, 12). Liver and adipose tissue are the two major sites of fatty acid synthesis in the rat (1); whereas the liver is the important site in the chicken (2).

Dietary carbohydrate is a major source of carbon for de novo fatty acid synthesis when animals are fed

high-carbohydrate diets. At least three factors are involved in the mechanism whereby diet composition affects de novo fatty acid synthesis:

(1) The rate at which the carbohydrate is presented to the tissue, which is governed in part by the rate of digestion and absorption of the carbohydrate.

(2) The manner or pathway in which different carbohydrates are metabolized by the tissue, and

(3) The intermediate metabolites, the availability of cofactors, the hormone status and the level of enzyme activities in the tissue are all involved in the regulation of fatty acid synthesis.

When diets containing glucose as the only source of carbohydrate are fed to rats three rate controlling enzymes in the glycolytic pathway, hexokinase, phosphofructokinase and pyruvate kinase are important in the regulation of glucose metabolism. In the rat liver, a high Km hexokinase (glucokinase) predominates and it has been suggested that glucose phosphorylation is the first controlled step in hepatic glucose metabolism (7). Recent studies indicate that the activities of phosphofructokinase and pyruvate kinase may be markedly altered in the rat by hormone administration (13, 14, 15, 16). But only a few studies have dealt with the regulation of glycolysis in chicken liver. Glucokinase is absent in chicken liver (17)

suggesting that glucose phosphorylation may be controlled by a different mechanism in this species.

The nutritional, enzymatic and hormonal status of the animal will influence the regulation of a metabolic pathway (1). However, the responses to these factors may vary among species. Comparison of the effects of various dietary carbohydrates on the regulation of lipid metabolism in various species should provide information for a better understanding of how dietary carbohydrate affects the regulation of lipid metabolism in humans and how our health might be improved.

Effect of Dietary Fructose on Glycolytic and Lipogenic Enzymes and on Fatty Acid Synthesis

In Normal Rats and Chickens

Fructose is rapidly removed from the portal vein by the liver, possibly because of the livers high fructokinase activity. Moreover, fructose is metabolized by the liver to dihydroxyacetone phosphate and glyceraldehyde and then may proceed through the glycolytic sequence to pyruvate which by passes the rate limiting glucokinase and phosphofructokinase steps. This direct fructolysis from fructose-1-P leads to an increase in α-glycerophosphate, which may increase the formation of triglyceride from fatty acyl CoA (8, 18).

In contrast to rat liver, rat adipose tissue lacks fructokinase activity and must rely on type (II) hexokinase to phosphorylate the fructose utilized by this tissue. Fructose feeding increased the activities of hepatic malic enzyme, citrate cleavage enzyme, fatty acid synthetase and acetyl CoA carboxylase in the rat; whereas the activities of these enzymes and hexokinase in adipose tissue were depressed by dietary fructose in comparison to glucose (9, 19). Dietary fructose stimulated hepatic fatty acid synthesis in the rat to a greater extent than did dietary glucose, but in adipose tissue fatty acid synthesis was depressed when fructose was fed. These observations are consistent with the concept that fructose is metabolized largely by the liver and that the circulating insulin levels are depressed by fructose feeding (8). Insulin is involved in regulating glucose uptake and the activity of fatty acid synthetase in rat adipose tissue. Although fructose caused a shift in the site of fatty acid synthesis, the total capacity of the rat to synthetize fatty acid was not influenced by fructose feeding (1, 20).

Our laboratory recently demonstrated that in contrast to observations in rats, dietary fructose did not stimulate the rate of fatty acid synthesis in the chicken liver (9). Liver is the predominant site of fatty acid synthesis in the chicken. Pearce (10) has also shown that the activities of several lipogenic enzymes such as

hepatic malic enzyme, citrate cleavage enzyme, acetyl CoA carboxylase and fatty acid synthetase were not elevated when the chickens were fed fructose rather than glucose. Pearce found that the fructokinase activity in chicken liver was elevated with fructose feeding. Since chicken liver lacks glucokinase and its fructose phosphorylation activity did increase when fructose was fed, the regulation of the initial steps of the glycolytic pathway may be different from those observed in rat liver. Apparently, the lipogenic response to fructose-containing diets depends on the species examined.

In Diabetic Rats

Fatty acid synthesis is regulated by the nutritional, enzymatic and hormonal status of the animal (1). Studies on the interrelationships between hormonal status and enzyme activities are now an active area of research. Insulin is an important hormone which has been suggested to be required for the dietary induction of rat hepatic malic enzyme, citrate cleavage enzyme, acetyl CoA carboxylase and fatty acid synthetase (21, 22). Dietary fructose, but not dietary glucose, can elevate fatty acid synthetase (23) and citrate cleavage enzyme activities (24) to normal value in diabetic rat liver but not adipose tissue. Thus, insulin probably is not required for high activity of certain lipogenic enzymes in rat liver but appears to be needed in adipose tissue. It is also possible that the

regulation of fatty acid synthetase depends on the concentration of certain intermediates of glycolytic pathway (23). Further work should be done in order to obtain a better understanding of the effect of dietary and hormonal factors on the regulation of metabolism.

Dietary Glycerol

Because of the potential for rather extensive use of glycerol to maintain water activity at relatively low levels during processing of intermediate moisture food-products, it seemed desirable to study the metabolic effects of dietary glycerol. These types of foods may contain more than 20% of free glycerol. This quantity of dietary glycerol greatly exceeds the total glycerol content of normal diets (approximately 2% of the diets as glyceride-glycerol). Glycerol is known to enter the glycolytic pathway at the same point as fructose does, however less is known of the metabolic effects of glycerol. The metabolic response obtained when fructose is fed depends on the species, as well as the organ, examined (1, 2, 20). Few studies have been conducted with dietary glycerol except in the rat (3, 6, 25, 26, 27, 28, 29, 30, 31, 32).

The Physiological Action of Glycerol

Theoretically, if combustion of glycerol was complete the caloric yield would be slightly greater than that of an equal weight of glucose. It is probable that some

of the ingested glycerol will be excreted in the urine either as glycerol or in modified form (31). In a prolonged experiment (40 weeks), it was shown that substitution of 40% glycerol for starch in a rat diet did not influence body weight gain (31). Interestingly, it was noticed that the rats fed 40% glycerol drank 30% more water than those fed the high starch diet. Rats fed the glycerol-containing diet were also observed to have diuresis. It has been suggested that glycerol can be substituted isocalorically for other carbohydrates in the diets of diabetic patients. Glucosuria and ketosis were decreased in diabetics given glycerol rather than glucose in their diets (32, 33).

The available evidence strongly suggests that glycerol transport is not a limiting process in glycerol utilization (34, 35). Therefore the rate of glycerol metabolism within the tissue must regulate glycerol uptake. There are three possible pathways of glycerol metabolism in the liver:

(1) phosphorylation to L-α-glycerophosphate by glycerol kinase;

(2) formation of L-α-glycerophosphate by pyrophosphate glycerol phosphotransferase;

(3) oxidation to glyceraldehyde by alcohol dehydrogenase.

The Km values for glycerol of pyrophosphate glycerol phosphotransferase and alcohol dehydrogenase are 3.0 M

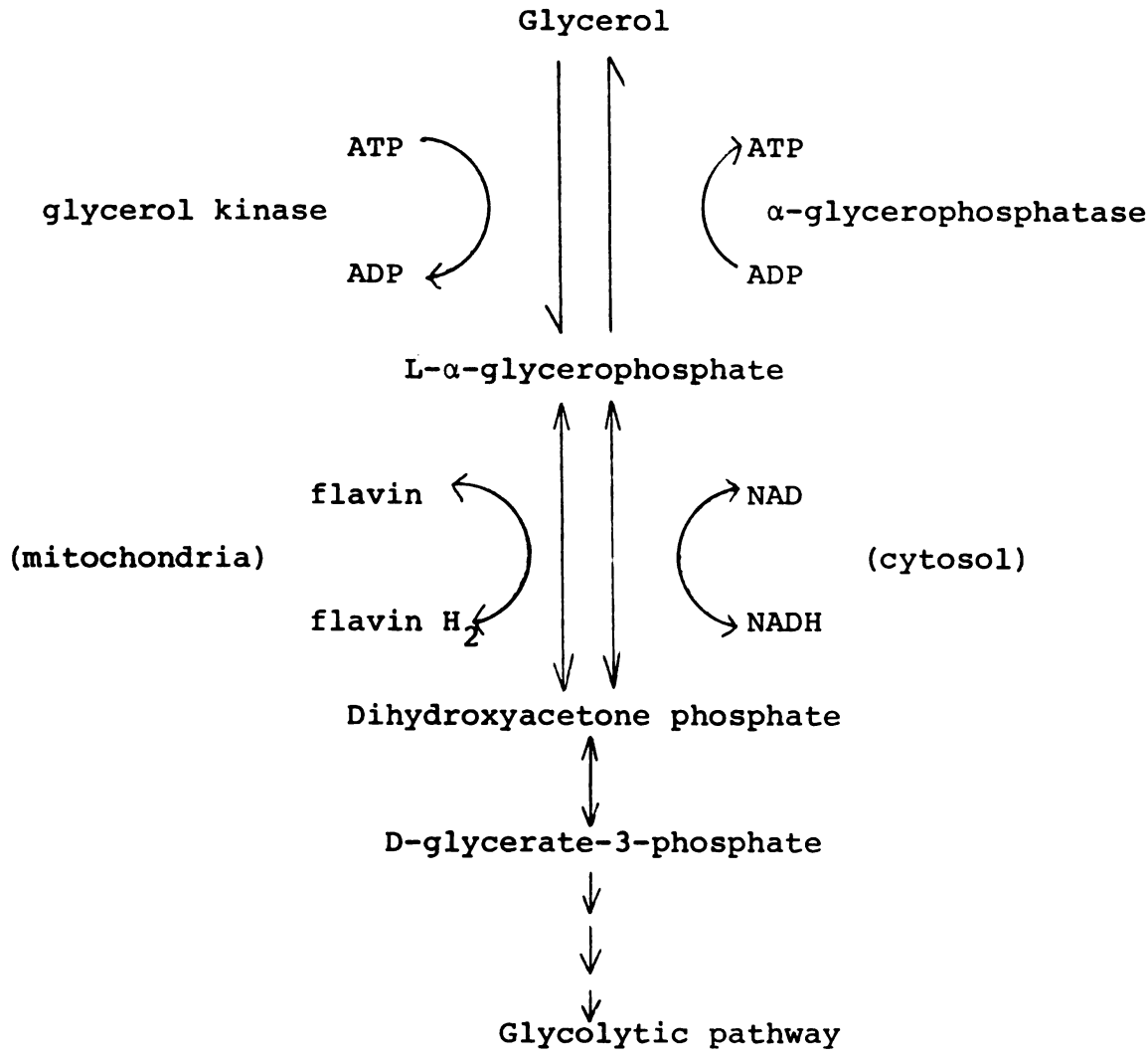

Fig. 1.--The pathway of glycerol metabolism.

(36) and 0.63 M (37), respectively. The Km of glycerokinase is 3 uM - 35 uM (38, 39, 40), which corresponds to the physiological level of glycerol in the liver. This suggests that glycerol is metabolized mainly through glycerokinase. Glycerokinase is a cytoplasmic enzyme and its activity is high in the liver and kidney, but low in the adipose tissue, intestine and other organs (41, 42, 43, 44), Glycerokinase is present in the epididymal adipose tissue of normal rats, as well as in brown fat (44, 45). In addition, Persico et al. (43) also concluded that insulin is involved in stimulation of glycerokinase activity in adipose tissue. But the activity of glycerokinase found in adipose tissue was low when compared to the activity found in the liver.

Glycerol Metabolism

In Rat Liver

The kinetic properties of glycerol kinase from rat liver with regard to ATP are apparently independent of variation in concentration of glycerol. The kinetics in regard to ATP can be described in terms of Michaelis-Menten mechanism only if the concentration of $ATP\text{-}Mg^{2-}$ is used instead of the total ATP concentration (38). The $ATP\text{-}Mg^{2-}$ as a true substrate for enzyme seems to be very similar to the mechanism involved in phosphoglycerate kinase (46). Feeding of a high fat diet resulted in an

increased liver glycerokinase activity. The components of fat responsible for the enhanced activity were fatty acids, not glycerol (47). Glycerol stabilizes the enzyme activity in vitro (41), but it did not increase the enzyme level when fed to rats. Glycerol uptake by rat liver slices was increased when the glycerol concentration was raised from 0.2 mM to 2.5 or 5.0 mM (48).

The pathway of glycerol metabolism via the Embden-Meyerhof's route in the liver is catalyzed by two enzymes, glycerokinase and α-glycerophosphate dehydrogenase, acting successively and producing dihydroxyacetone phosphate. Burch et al. found that α-glycerophosphate dehydrogenase activity in the liver is far more active than glycerol kinase (49). With high levels of glycerophosphate, the dehydrogenase would be expected to produce a corresponding increase in dihydroxyacetone phosphate or NADH:NAD^+ ratio. However, neither of two cases occurred (49). Two years before Burch et al. published their study, the Teppermans had shown an interesting result that feeding glycerol rather than glucose increased the activity of glycerophosphate dehydrogenase and slightly decreased the activity of glycerokinase in rat liver (3). This result was similar to that obtained by Cryer and Bartley, who found no effect of 53.4% glycerol diet on the activity of glycerol kinase but a significant elevation in α-glycerophosphate dehydrogenase after feeding a 53.4%

glycerol diet for 20 days (5). It is possible that glycerol uptake could be altered by a change in activity of NAD-linked cytoplasmic α-glycerophosphate dehydrogenase which could modify glycerol kinase activity through an effect on α-glycerophosphate. In E. coli glycerokinase is inhibited by high concentrations of α-glycerophosphate (50). If mammalian glycerokinase is regulated in a manner similar to that found in the E. coli hepatic phosphorylation of glycerol might be the rate-limiting step in glycerol metabolism (3, 4).

Glycerol, like fructose, is metabolized to "instant triose" very rapidly in vivo. It remains to be established what role these "instant trioses" play in the regulation of lipogenesis in vivo. Once the glycerol and fructose have been converted into triose phosphate, both the pathways of glycolysis and gluconeogenesis are available. Takeda et al. reported that glycerol feeding caused an elevation in the activities of all the key enzymes related to glucose degradation including pyruvate kinase, glucose-6-P dehydrogenase, ATP citrate lyase and acetyl-CoA carboxylase. However, glycerol feeding did not affect the gluconeogenic pathway in normal rat liver (6). Whereas, Sillero et al. (27) have reported conflicting finding that glucose-6-phosphatase activity, which is a gluconeogenic enzyme above the triose phosphate level, was increased by feeding 60% glycerol to rats for 3 days. Fructose feeding

or glycerol feeding results in a greater production of α-glycerophosphate than does the ingestion of glucose, and this increased level of α-glycerophosphate may be in part responsible for the increased level of serum glycerides observed when fructose or glycerol are fed (24, 26, 28, 29, 51).

Some of the pathways of glucose, fructose and glycerol metabolism can be summarized as follows (Figure 2). A high concentration of α-glycerophosphate probably could stimulate fatty acid synthesis by removing acyl CoA esters, indirectly by allosteric activation of acetyl CoA carboxylase or by acting directly or indirectly as an inducer of the enzymes of fatty acid synthesis in vitro (52, 53, 54). However, not only is the hepatic concentration of α-glycerophosphate not directly correlated with fatty acid synthesis but instead is inversely related in vivo (55).

In Rat Adipose Tissue

Recent studies suggest that glycerokinase is present in rat adipose tissue. Insulin is involved in the stimulation of glycerokinase activity (43). This glycerokinase activity in adipose tissue is sufficient to result in glycerol utilization that is considerably higher than previous thought (44, 56).

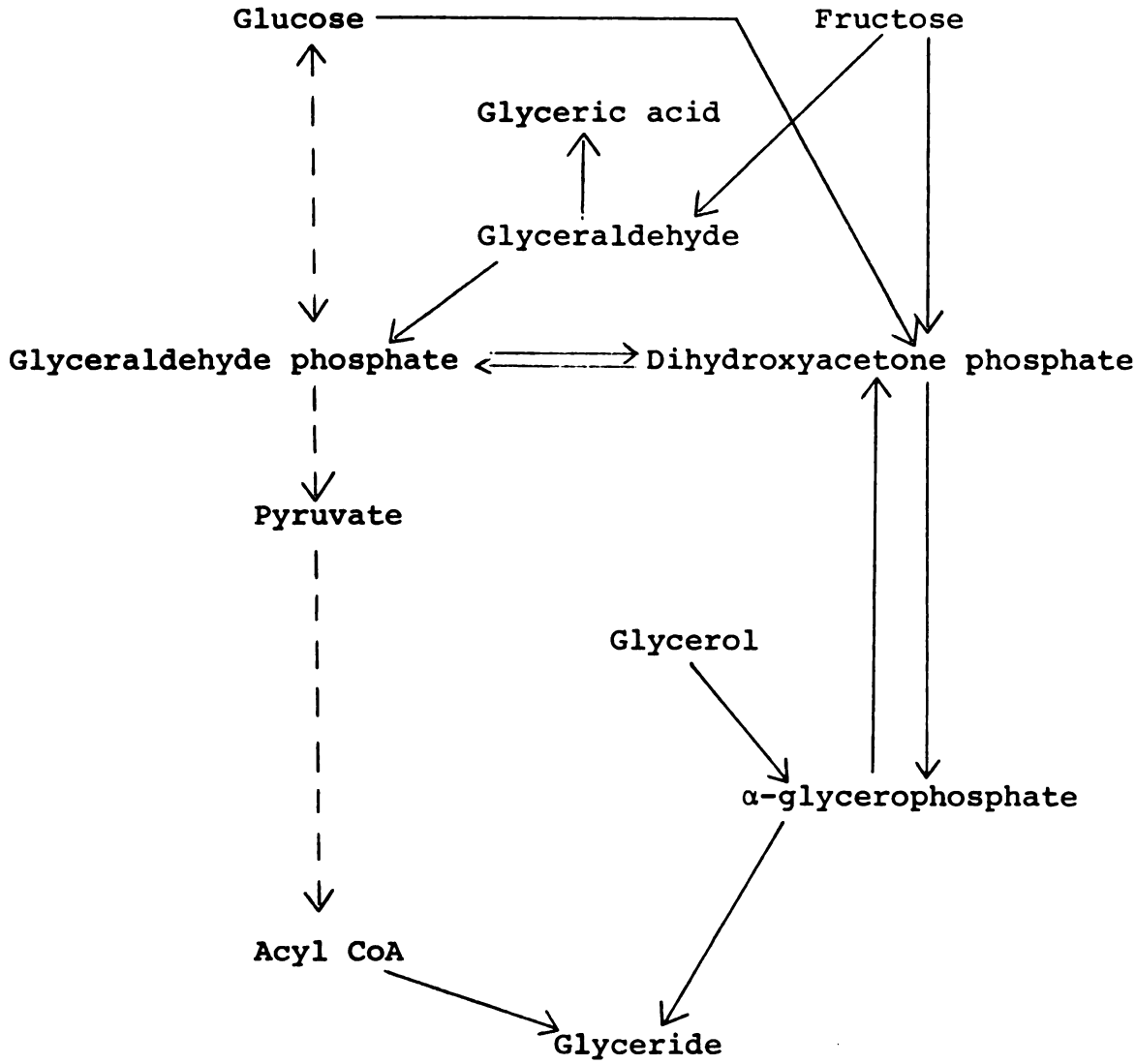

Fig. 2.--Several pathways of glucose, fructose and glycerol metabolism.

In Chicken Liver

Few studies have been conducted on the influence of glycerol on metabolism. Hepatic glycerokinase activity was stimulated in the chicks fed either high fat or high protein diets, whereas hepatic α-glycerophosphate dehydrogenase activity was not influenced by the diet fed (57).

In Chicken Adipose Tissue

The site for fatty acid synthesis appears to differ between the avian and mammalian species. The liver, not adipose tissue, is responsible for about 90% of de novo fatty acid synthesis in the chicken (2, 58). Glycerokinase is present in chicken adipose tissue and can be altered by dietary manipulation (59).

Influence of Dietary Glycerol on the Enzyme Involved in Lipogenesis and on the Rate of Fatty Acid Synthesis

It is known that normal rats fed a high-glycerol diet exhibit a marked elevation in the activities of glucose-6-phosphate dehydrogenase, malic enzyme, citrate cleavage enzyme and acetyl CoA carboxylase, enzymes involved in hepatic lipogenesis (3, 6, 27, 28). Administration of a high-glycerol diet to diabetic rats also cause a dramatic induction of citrate cleavage enzyme and acetyl CoA carboxylase activities (6). As previously indicated, certain lipogenic enzymes in rat liver are not directly influenced by insulin, but they depend on the

degree of carbohydrate utilization. Metabolism of glycerol by normal and diabetic liver has been investigated by Ashmore et al. (60). They reported that glycerol metabolism occurred very rapidly and its utilization was not impaired in diabetic animals. Therefore, glycerol can be substituted isocalorically for glucose to decrease glucosuria and ketosis in diabetic animals (31, 32, 33). Though glycerol feeding augments the activities of key enzymes involved in hepatic lipogenesis, evidence is still lacking on the influence of glycerol on fatty acid synthesis in rat liver.

Current research orientation has placed major emphasis upon the effects of dietary glycerol on hepatic cholesterol and triglyceride level (29). It is possible that an excess α-glycerophosphate from glycerol feeding leads to increase in serum glycerides (26). Early workers indicate that the feeding of glycerol to humans and animals has not shown any major ill effects (31). More recently, several investigators observed that normal subjects consuming glycerol exhibited enhanced alimentary hypertriglycerdemia (26, 30) while reports on the beneficial properties of glycerol feeding have been largely confined to studies in rats (5, 6, 28). Other findings reported that a high-glycerol diet enlarged liver, elevated hepatic lipids, especially triglycerides, and elevated

lipogenic enzyme activities (29). Similar studies in chickens have not been reported yet.

In conclusion, a differential response to nutritional alterations such as glucose and glycerol feeding would be expected. An exploration of such responses should be very interesting. The specific focus of my research was on the influence of glycerol on metabolism in animal models. Parameters studied were body weight, liver and epididymal adipose weight, food consumption and activities of fatty acid synthetase, citrate cleavage enzyme, and malic enzyme and rates of fatty acid synthesis. Because rats and chickens are two animal models which differ significantly in several aspects of lipogenesis, a comparison with each other should provide an increased understanding of the effect of diet on lipogenic enzymes activities and on rates of fatty acid synthesis. The specific experiments to be reported here were designed to:

(1) establish the in vitro effect of glycerol on fatty acid synthesis in livers of rats and chickens and in adipose tissue of rats;

(2) investigate the in vivo effect of dietary glycerol on body weight gain, on the activities of fatty acid synthetase, citrate cleavage enzyme, malic enzyme and on the rates of fatty acid synthesis in rats and chickens.

Liver is the major site of fatty acid synthesis in man as well as in chicken (2, 11, 12, 58). The influence of dietary glycerol on fatty acid synthesis in chicken liver may contribute to our understanding of glucose metabolism. Based on current evidence (3, 6, 27) we would predict that glycerol feeding would stimulate rates of fatty acid synthesis in rat liver but not in rat adipose tissue. Results from these series of experiments with chickens and rats should provide information useful for an increased understanding of the mechanisms involved in the dietary regulation of lipid metabolism.

EXPERIMENTAL

Materials

Animals and Diets

The experiments were conducted with male Sprague-Dawley rats and male crossbred heavy type chickens. Diets and water were provided at all time unless indicated otherwise. Diets were prepared on an equal energy basis (Table 1).

Housing

Rats were housed individually in stainless steel cages having raised wire floors. Chickens were generally housed in groups of five and in heated cages having raised wire floors. The chicks were wing-banded, and, upon reaching an average weight of 80 g (Experiment IV) or 400 g (Experiment V), they were assigned to one of three or one of two treatment groups respectively.

Chemicals and Reagents

Krebs-Ringer Bicarbonate Solution.--The solution was modified from original (61) by addition of (a) 0.1 unit of porcine insulin per ml of buffer, (b) 40 uCi of tritiated water per ml of buffer, (c) different substrate

Table 1.--Composition of diets.

	Species					
	Rat			Chicken		
	parts					
Glycerol[a]	0	20.0	40.0	0	20.0	40.0
Glucose[b]	66.1	43.6	21.0	48.66	26.91	3.7
Casein	20.0	20.0	20.0	--	--	--
Soybean meal protein	--	--	--	30.0	30.0	30.0
Isolated soy protein	--	--	--	6.0	6.0	6.0
Mineral mixture[c]	4.0	4.0	4.0	5.31	5.31	5.31
Fiber	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mixture	0.4	0.4	0.4	0.4	0.4	0.4
Methionine	0.3	0.3	0.3	0.33	0.33	0.33
Choline chloride	0.2	0.2	0.2	0.3	0.3	0.3
Corn oil	5.0	5.0	5.0	5.0	5.0	5.0
TOTAL	100.0	97.5	94.9	100.0	98.25	95.04
Calculated metabolizable energy[d]	3.66	3.75	3.85	3.61	3.70	3.80

[a]Estimated metabolizable energy is 4.10 Kcal per gram (62).

[b]Estimated metabolizable energy is 3.64 Kcal per gram (62).

[c]Different mineral mixture for the rat (63) and the chicken (64).

[d]Values are expressed as Kcal per gram of feed.

which was indicated in the methods. The buffer was gassed with a 5% CO_2-95% O_2 mixture for 10 minutes. The pH of the buffer was 7.4.

Biochemicals.--The cofactors CoA, and ATP as well as NADH and NADPH were ordered from Sigma Chemical Co., St. Louis, Missouri. Acetyl CoA and malonyl CoA were purchased from P. L. Biochemicals, Milwaukee, Wisconsin. Malate dehydrogenase was obtained from Boehringer Mannheim.

Hydrogen-Labeled Isotopes.--Tritiated water was obtained from New England Nuclear, Boston, Massachusetts.

Scintillation.--Omniflour, a blend of 98% PPO and 2% Bis-MSB, was ordered from New England Nuclear, Boston, Massachusetts.

Methods

In Vitro Studies

1. Male Sprague-Dawley rats (Spartan Research Animals, Haslett, Michigan, U.S.A.) weighing about 70 g to 160 g were killed by decapitation after feeding the high-glucose basal diet (Table 1) for 1 - 2 weeks. Other groups of rats were fasted overnight before killing to lower liver glycogen levels. Glycogen is a major carbon source for de novo fatty acid synthesis in liver slices (65). Liver slices (100-150 mg) were prepared with a

Stadie-Riggs hand microtome immediately after killing the animals. Liver slices and pieces of distal epididymal fat pads weighing about 120 mg were incubated for 2 hours in 3 ml Krebs-Ringer bicarbonate buffer containing 40 uCi $^{3}H_2O$ and 0.1 unit porcine insulin per ml and different substrates indicated as follows: basal (no exogenous substrate), 10 mM glucose, 1 mM glycerol, 3 mM glycerol and 10 mM glycerol. In order to obtain further information on the effect of glycerol on hepatic fatty acid synthesis, liver slices were also incubated in a buffer containing 10 mM acetate with or without 3 mM glycerol or 10 mM pyruvate with or without 3 mM glycerol. After incubating at 37° in a Dubnoff metabolic shaker which was maintained at an atmosphere of 5% CO_2-95% O_2 for 2 hours, the tissues were removed and rinsed several times in 0.9% saline and blotted lightly on filter paper to remove any of the radioactive buffer. Then the tissues were transferred to tubes containing 3 ml of 30% potassium-hydroxide and 7-8 ml of 95% ethanol. The tissues were saponified at 70-80° for 2 hours. Marbles were placed on the tops of the tubes to reduce evaporation. After the samples had been saponified, the tubes were cooled to room temperature and 10 ml of distilled water was added to each tube. The nonsaponifiable lipid fraction was extracted with two 5 ml portions of petroleum ether for liver slices only. The aqueous phase was acidified with concentrated hydrochloric acid until

congo red paper turned blue. Extraction procedure was accomplished by adding 5 ml of petroleum ether to the tubes, capping them, and mixing each tube on a Vortex Mixer. After that, the samples were set aside until the organic and aqueous phases in the tubes had separated. The organic phase which contains radioactive fatty acids were removed into glass scintillation vials. The fatty acids were extracted into vials by 3 successive 5 ml of petroleum ether (66). The scintillation vials were uncovered and put in a forced-draft hood overnight to evaporate all of the ether. Scintillation cocktail was prepared by addition of 4.0 g of omniflour, 230 ml of ethyl alcohol and diluted to 1 liter with tolulene.

2. Male crossbred heavy type chickens (Nussbaum Hatchery, Remington, Ind.) were fed a commercial diet (Master Mix Pullet grower, Central Soya, Fort Wayne, Ind.) for 1 week. Chickens weighed about 300 g and were killed by cervical dislocation. The liver were sliced immediately with a Stadie-Riggs hand microtome. The incubation buffers were similar to those described for the rat experiments. The only difference was that the glycerol concentration was increased to 25 mM in one buffer. The methods for isolating and counting radioactive fatty acids were as above (66).

In Vivo Studies

Five experiments were conducted to determine the effect of dietary glycerol on body weight gain, lipogenic enzyme activities and rates of fatty acid synthesis in rats and chickens. Experiment I was conducted in rats for 3 days period; experiment II and III were conducted in rats fed the experimental diets for 3 weeks. Experiment IV and V were conducted in chickens fed the experimental diets for 3 days and 3 weeks, respectively. Individual body weights were recorded weekly. Individual food consumption was recorded in the rat experiments, while group food intakes were recorded in the chicken experiments. On the day of killing, rats were injected intraperitoneally with ^3H_2O and chickens were injected in the wing vein with ^3H_2O. The use of ^3H_2O estimates the rate of fatty acid synthesis independent of substrate (67). The dose of ^3H_2O was about 690 uCi per 100 g of body weight and 290 uCi per 100 g of body weight for rats and chickens, respectively. Rats were killed 15 minutes and chickens were killed 3 minutes after injection. We assumed that translocation of labeled fatty acids from one compartment to another was minimal during these short time intervals.

Homogenization of Tissues

The livers of rats or chickens and the left and the right epididymal fat pads of rats were rapidly excised. The tissues were rinsed in ice-cold 0.9% saline, gently

blotted dry on paper towels and weighed. Buffer containing 0.15 M KCl, 1 mM $MgCl_2$ and 0.01 M N-acetyl cystein pH 7.6, 8 ml of buffer per g tissue was used to homogenize each sample. The supernatant was used for assay of enzyme activities.

Assay of Enzyme Activity

The activities of fatty acid synthetase (68), citrate cleavage enzyme (69) and malic enzyme (70) were measured at 25° or at 37° by either an increase or decrease in absorbance of the reaction mixture at 340 nm.

Fatty Acid Synthetase.--The assay mixture contained 1 mM KH_2PO_4, pH 6.5, 10 mM EDTA, 100 mM acetyl cysteine, 0.3 mg bovine serum albumin, 0.2 mM acetyl CoA, 0.75 mM NADPH, 0.5 mM malonyl CoA and 10-20 ul of either liver or epididymal fat pad supernatant in a final volume of 1 ml. All ingredients were mixed together except malonyl CoA. The blank was distilled water. The reaction was initiated by addition of malonyl CoA and absorbance change was recorded for 3 minutes. Malonyl CoA and NADPH were prepared on the day of use.

Citrate Cleavage Enzyme.--The reaction mixture contained 0.5 M Tris buffer, pH 8.4, 0.2 M $MgCl_2$, 0.2 M N-acetyl cysteine, 0.2 M K-citrate, 0.1 M ATP, 10 mM NADH, 20 units per ml of malate dehydrogenase, 2 mM CoA, and 10 ul of either liver or epididymal fat pad

supernatant in a final volume of 1.1 ml. Everything was added except CoA. The reaction was started by the addition of CoA. The ATP and NADH were prepared daily in water.

Malic Enzyme.--For the assay of malic enzyme activity, 0.05 M manganese chloride, 0.284 M gly-gly buffer, pH 7.4, 0.22 mM NADP and 10-20 ul of either liver or adipose supernatant were mixed to a final volume of 2.8 ml. The reaction was started by addition of 111.8 mM freshly prepared L-Malate pH 7-8 to the assay mixture. Absorbance was recorded for 3 minutes.

Protein Determination.--Protein concentration were determined by a slight modification of the method of Lowry et al. (71). A stand curve was prepared from 25 ug to 100 ug of bovine serum albumin. If necessary, liver or epididymal fat pad supernatants were diluted with water, so as to adjust the protein concentration within the standard curve range. Unknown protein samples were performed in duplicate and read at 500 nm.

Specific Activity.--Enzyme specific activities were expressed as the amount of enzyme which catalyzes the utilization of one nanomole of NADPH, NADP or NADH per mg of protein per minute at 25 or 37°.

In Vivo Fatty Acid Synthesis

The remaining part of the liver were subsequently homogenized with equal weight of distilled water, and duplicate 0.5 ml aliquots of the homogenate were saponified. Duplicate samples of distal portions of rat adipose tissue, weighing about 150 mg, were also saponified. Saponification, extraction and counting of fatty acids were done according to procedures described above (66).

RESULTS

In Vitro Experiments

The results of experiments to determine the effect of substrate concentration on rates of in vitro fatty acid synthesis in fed and fasted rats are presented in Table 2. The rates of fatty acid synthesis in liver slices from rats fed a high-glucose diet were not increased when 10 mM glucose was added to the buffer. Buffer containing 1 mM glycerol did not influence the rates of fatty acid synthesis; however, buffer containing 3 mM or 10 mM glycerol depressed the rates of tritium incorporation into hepatic fatty acids by approximately 50%.

Endogenous glycogen is the major carbon source for de novo fatty acid synthesis in liver slices from fed rats (65). In order to see if glycerol would stimulate fatty acid synthesis in the absence of endogenous glycogen rats were fasted overnight prior to killing to deplete the glycogen. In contrast to the observations observed in liver slices from fed rats, an increase in fatty acid synthesis was observed when 10 mM glucose was added to the buffer. However, glycerol-containing buffer did not increase the rate of tritiated water incorporation into

Table 2.--In vitro effect[a] of glycerol on fatty acid synthesis in rat liver and adipose tissue.

Group	Source of Tissue	Carbohydrate Concentration (mM)				
		Basal	Glucose	Glycerol		
		0	10	1	3	10
Fed	Liver	304±52	330±52	285±55	169±23	162±19
P value[b]		NS		NS	0.02	0.02
Fasted	Liver	67±9	102±11	71±8	78±13	74±8
P value[b]		0.025		0.05	0.10	0.05
Fed	Adipose	--	7922±400	--	--	477±43
P value[b]						0.001

[a]Values are means ± SEM for 9 fed rats and 10 fasted rats. Rats were fed a high-glucose diet for 2 weeks or fasted overnight prior to killing. Results are expressed as dpm ^3H_2O incorporated into fatty acids per 100 mg tissue per 2 hours.

[b]Probability that the observed value is significantly different from the value obtained when the buffer contained 10 mM glucose. NS = non-significant.

fatty acids in liver slices of fasted rats. Thus, the rate of fatty acid synthesis was greater when 10 mM glucose was added to the buffer than when 3 or 10 mM glycerol was added to the buffer.

In vitro estimates of adipose fatty acid synthesis in rats were markedly lower when glycerol, rather than glucose was added to the buffer.

To obtain some information on the inhibitory effect of glycerol on hepatic fatty acid synthesis, liver slices were incubated with pyruvate or acetate either in the presence or absence of 3 mM glycerol (Table 3). There were no significant differences in the rate of fatty acid synthesis when 3 mM glycerol was added to either the 10 mM acetate-containing or the 10 mM pyruvate-containing buffer. This suggests that the inhibitory action of glycerol occurs at the level of glycogen or glucose conversion to pyruvate.

The influence of glycerol on the rates of fatty acid synthesis in chicken liver slices is presented in Table 4. The chickens, in contrast to rats, did not show any significant difference of the rates of fatty acid synthesis when 10 mM glycerol, or even 25 mM glycerol, was added to the incubation buffer. The results obtained in this experiment are independent of choice of substrate as well as substrate concentration used. From Table 2 and Table 4, it suggested that the in vitro effect of glycerol on the rates of fatty acid synthesis has a more dramatic

Table 3.--Effect of glycerol on the rate of fatty acid synthesis in liver slices incubated with pyruvate or acetate.[a]

	Substrate Concentration (mM)			
	10 mM Pyruvate		10 mM Acetate	
	No Glycerol	3 mM Glycerol	No Glycerol	3 mM Glycerol
Fatty acid synthesis[b]	1501±195	1407±180	1131±169	1328±145
P value[c]		NS		NS

[a]Values are means of the 10 rats ± SEM. The rats were fed a high-glucose diet for 1 week.

[b]Fatty acid synthesis expressed as dpm ^3H_2O incorporated into fatty acids per 100 mg liver per 2 hours.

[c]Probability of significant treatment effect; NS indicates not significant.

Table 4.--In vitro effect of glycerol on fatty acid synthesis in chicken liver.[a]

	Carbohydrate Concentration (mM)				
	Basal	Glucose	Glycerol		
	0	10	3	10	25
Fatty acid synthesis[b]	3327±497	4442±478	4074±393	3681±402	3831±300
P value[c]	NS		NS	NS	NS

[a]Values are the means of the 10 chickens ± SEM. The chickens were fed a high-carbohydrate diet for 10 days.

[b]Fatty acid synthesis expressed as dpm $^{3}H_2O$ incorporated into fatty acids per 100 mg tissue per 2 hours.

[c]Probability of significant treatment effect; NS indicates not significant.

influence on rat liver than on chicken liver. The reason for this differential response in rats and chickens is not clear.

In Vivo Experiments

Body Weight, Food Intake, Liver Weight and Epididymal Fat Pad Weight

The glycerol-containing diet did not dramatically affect body weight gain, liver weight and adipose weight in rats or chickens fed the diets (Table 1) for 3 days. Food consumption was not significantly influenced when the rats were fed the glycerol-containing diets rather than the high-glucose diet for 3 days. However, chickens fed the 20-parts glycerol diet consumed more energy than did chickens fed the high-glucose diet for 3 days (Table 5).

Rats and chickens were also fed the experimental diets for 3 weeks (Table 6). Food intake was significantly depressed in the rats, but unchanged in the chickens, when the 20-parts glycerol diet was fed for 3 weeks. Substitution of 20-parts glycerol for glucose in the diet did not significantly affect body weight gain of either rats or chickens (Table 6). But when 40-parts glycerol was added to the diet the body weight gain and food intake were significantly depressed in both rats and chickens (Table 6). Liver weights of rats were significantly heavier in the rats fed the glycerol-containing diets for 3 weeks than in

Table 5.--Effect of dietary glycerol on body weight gain, total food intake and liver weight in rats and chickens and on adipose weight in rats fed the diets for 3 days.[a]

	Dietary Glycerol - Parts			
	Rat (Experiment I)		Chicken (Experiment IV)	
	0	20.0	0	20.0
Final wt. (g)	179±2	176±3	618±9	655±16
Body wt. gain (g)	40±1	36±1	161±9	186±11
Food consumption (Kcal)	213±10	200±7	892±7	1075±14[b]
Liver wt. (g tissue/ 100 g body wt.)	4.9±0.1	5.0±0.1	3.8±0.1	3.7±0.1
Adipose wt. (g tissue/ 100 g body wt.)	0.56±0.03	0.55±0.04	--	--

[a]Values are the means of 10 animals ± SEM. Rats and chickens were fed either high-glucose or 20-parts glycerol diets (Table 1) for 3 days.

[b]Significantly different from the high-glucose diet group at $p < 0.001$.

Table 6.--Effect of dietary glycerol on body weight gain, total food intake, liver weight in rats and chickens and on adipose weight in rats fed the diets for 3 weeks.[a]

Species	Experiment		Dietary Glycerol - Parts		
			0	20.0	40.0
Rat	II	Final wt. (g)	229±4	227±3	188±3[b]
		Body wt. gain (g)	157±3	154±2	114±3[b]
		Food intake (Kcal)	1712±34	1595±19[b]	1331±37[b]
		Liver wt. (g tissue/ 100 g body wt.)	4.6±0.4	5.8±0.1[b]	6.2±0.2[b]
		Adipose wt. (g tissue/ 100 g body wt.)	1.04±0.06	1.12±0.04	0.91±0.03[b]
	III	Final wt. (g)	239±6	233±6	--
		Body wt. gain (g)	146±5	139±4	--
		Food intake (Kcal)	1545±37	1423±27[b]	--
		Liver wt. (g tissue/ 100 g body wt.)	5.06±0.05	5.64±0.07[b]	--
		Adipose wt. (g tissue/ 100 g body wt.)	0.98±0.04	0.93±0.04	--
Chicken	V	Final wt. (g)	636±17	624±19	345±16[b]
		Body wt. gain (g)	557±16	545±20	265±16[b]
		Food intake (Kcal)	3427±70	3599±43	2073±45[b]
		Liver wt. (g tissue/ 100 g body wt.)	3.5±0.1	3.5±0.1	3.8±0.1

[a]Values represent mean ± SEM for 10 animals per treatment except only 8 chickens were fed the diet containing 40-parts glycerol.

[b]Significantly different from the high-glucose diet group at $p < 0.05$.

the glucose-fed rats (Table 6). Unlike in the rats, chicken liver weights were not influenced by addition of glycerol to the diet. The weights of epididymal adipose in rats fed the diet containing 40-parts glycerol were significantly decreased (Table 6).

Hepatic Lipogenic Enzyme Activity and the Rates of Fatty Acid Synthesis in Rat

In the 3-day study the liver soluble protein content was increased significantly in the glycerol-fed rats. However, liver soluble protein, expressed as mg per g of liver, was not significantly different among the rats fed the different diets for 3 weeks. Hepatic activities of fatty acid synthetase, citrate cleavage enzyme, and malic enzyme were all significantly higher in glycerol than in glucose treated rats, whether the diets were fed 3 days or 3 weeks (Table 7). But the increased lipogenic enzyme activities observed did not lead to a stimulation in the rate of fatty acid synthesis (Table 7). In fact the in vivo rate of fatty acid synthesis in rat liver was significantly depressed in one of the two 3-week experiments when 20-parts glycerol replaced glucose in the diet (Table 7). The rate of fatty acid synthesis in livers of rats fed the 40-parts glycerol diet was similar to that in glucose-fed rats (Table 7).

Table 7.--Influence of glycerol feeding on lipogenesis in rats.[a]

Length of Feeding	Experiment		Dietary Glycerol - Parts		
			0	20.0	40.0
3 days	I	Liver soluble protein, mg/g liver	78±1	84±2[d]	--
		Fatty acid synthetase[b]	13±2	19±1[d]	--
		Malic enzyme[b]	32±2	49±4[d]	--
		Fatty acid synthesis[c]	294±45	350±73	--
3 weeks	II	Liver soluble protein, mg/g liver	96±3	103±4	97±6
		Fatty acid synthetase[b]	17±1	27±4[d]	22±2[d]
		Fatty acid synthesis[c]	182±30	96±15[d]	166±34
	III	Liver soluble protein, mg/g liver	84±7	96±2	--
		Fatty acid synthetase[b]	7.8±0.8	13.2±0.4[d]	--
		Citrate cleavage enzyme[b]	33±3	52±2[d]	--
		Malic enzyme[b]	14±2	35±2[d]	--
		Fatty acid synthesis[c]	284±45	233±39	

[a]Values are the means of 10 rats ± SEM.

[b]One unit of enzyme activity is defined as conversion of one nanomole of substrate to product per mg of protein per minute at 25° (Experiment I and II) or 37° (Experiment II).

[c]Fatty acid synthesis expressed as dpm ^3H_2O incorporated into fatty acids per 100 mg liver per 15 minutes.

[d]Significantly different from the high-glucose treated group at $p < 0.05$.

Lipogenic Enzyme Activity and In Vivo Rates of Fatty Acid Synthesis in Chicken Liver

The activities of several lipogenic enzymes were measured in chicken liver after feeding chickens glycerol-containing diets for 3 days or 3 weeks (Table 8). In the 3-day or 3-week study the liver soluble protein content was unchanged in chickens fed 20-parts glycerol-containing diets. But the soluble protein content in chickens fed the diet containing 40-parts glycerol for 3 weeks were significantly increased. Unlike in the rat liver, activities of fatty acid synthetase and citrate cleavage enzyme were not changed but malic enzyme activity decreased when chickens were fed glycerol-containing diet for 3 days. In chickens fed the diets for 3 weeks the activities of fatty acid synthetase and malic enzyme were significantly decreased with glycerol feeding. Especially in the group fed 40-parts glycerol fatty acid synthetase and malic enzyme activities were decreased dramatically. These chickens also consumed less diet than did other chickens (Table 6).

The rates of fatty acid synthesis were depressed in livers of chickens fed the glycerol-containing diets for 3 days or for 3 weeks. Glycerol feeding appeared to depress the rate of fatty acid synthesis in chicken liver prior to an observable change in hepatic fatty acid synthetase activity.

Table 8.--Influence of glycerol feeding on lipogenesis in chicken liver.[a]

Length of Feeding	Experiment		Dietary Glycerol - Parts 0	20.0	40.0
3 days	IV	Liver soluble protein, mg/g liver	86±2	85±3	--
		Fatty acid synthetase[b]	21±1	24±3	--
		Citrate cleavage enzyme[b]	78±4	74±5	--
		Malic enzyme[b]	184±9	153±9[d]	--
		Fatty acid synthesis[c]	169±11	115±12[d]	--
3 weeks	V	Liver soluble protein, mg/g liver	78±4	76±3	107±11[d]
		Fatty acid synthetase[b]	34±5	20±3[d]	3.3±0.7[d]
		Malic enzyme[b]	147±11	112±8[d]	5±1[d]
		Fatty acid synthesis[c]	123±14	88±10[d]	17±3[d]

[a]Values of chickens fed 40-parts of glycerol are the means of 8 observations ± SEM. Other values represent mean ± SEM for 10 chickens.

[b]One unit of enzyme activity is defined as conversion of one nanomole of substrate to product per mg of protein per minute at 25°.

[c]Fatty acid synthesis expressed as dpm $^{3}H_2O$ incorporated into fatty acids per 100 mg liver per 3 minutes.

[d]Significantly different from the high-glucose treated chickens at $p < 0.05$.

Adipose Lipogenic Enzyme Activity and In Vivo Rates of Fatty Acid Synthesis in Rat

Despite the emphasis on liver, the adipose tissue is also an important site of fatty acid synthesis in the rat (1). Unlike liver, adipose tissue contains low activity of glycerokinase. Results of the influence of dietary glycerol on lipogenesis in rat adipose tissue are presented in Table 9. The soluble protein concentration of adipose tissue from rats fed the glycerol-containing diet for 3 days was lower than observed in rats fed the glucose diets, however differences in soluble protein concentration were not observed in the 3 week experiments (Table 9). No significant changes in adipose activities of fatty acid synthetase, citrate cleavage enzyme and malic enzyme in rats fed glycerol-containing diet for 3 days or for 3 weeks were noted (Table 9). The rate of fatty acid synthesis in rat adipose tissue also was not influenced by glycerol feeding. Rather large variations in the rates of fatty acid synthesis within treatments were observed.

Table 9.--Activity of lipogenic enzymes and the rate of fatty acid synthesis in adipose tissue of rats fed diets containing glycerol.[a]

Length of Feeding	Experiment		Dietary Glycerol - Parts 0	20.0	40.0
3 days	I	Adipose soluble protein, mg/g adipose tissue	16±1	12±1[d]	--
		Fatty acid synthetase[b]	30±4	25±4	--
		Malic enzyme[b]	165±18	186±14	--
		Fatty acid synthesis[c]	656±172	335±96	--
3 weeks	II	Adipose soluble protein, mg/g adipose tissue	14±1	14±1	15±1
		Fatty acid synthetase[b]	38±9	39±6	35±3
		Fatty acid synthesis[c]	183±64	66±20	132±33
	III	Adipose soluble protein, mg/g adipose tissue	11±1	13±1	--
		Fatty acid synthetase[b]	30±4	23±2	--
		Citrate cleavage enzyme[b]	67±11	61±5	--
		Malic enzyme[b]	159±14	145±16	--
		Fatty acid synthesis[c]	220±53	146±29	--

[a]Values are the means of the 10 rats ± SEM.

[b]One unit of enzyme activity is defined as conversion of one nanomole of substrate to product per mg of protein per minute at 25° (Experiment I and III) or 37° (Experiment II).

[c]Fatty acid synthesis expressed as dpm $^{3}H_{2}O$ incorporated into fatty acids per 100 mg adipose tissue per 15 minutes.

[d]Significantly different from the high-glucose treated group at $p < 0.05$.

DISCUSSION

Dietary manipulations influence the metabolism of protein, carbohydrate, and lipid. Much of our information on the influence of metabolism comes from experiments with animal models.

It is now clear that there are tissue-specific responses to various dietary factors. The influence of dietary fructose and sucrose on lipogenic enzyme activity and fatty acid synthesis in rat liver and adipose tissue has been reported (1, 8, 10, 72, 73). Similar studies on the chicken have also been conducted (9, 10). Glycerol, as well as fructose, enters the glycolytic pathway at the triose phosphate level, however less is known of the metabolic effects of glycerol.

I have studied the influence of glycerol on fatty acid synthesis in rats and chickens. The rates of fatty acid synthesis in liver slices from fed rats were determined (Table 2). Buffer containing 3-10 mM glycerol depressed the rates of fatty acid synthesis. Clark et al. (65) have also presented evidence that glycerol at concentration above 5 mM inhibit the incorporation of ^{3}H from $^{3}H_2O$ into fatty acids in rat hepatocytes. Liver

slices from fed rats contain sufficient endogenous glycogen to maintain high rates of fatty acid synthesis in the absence of exogenous substrate. To examine the influence of glycerol on fatty acid synthesis in liver slices low in glycogen, rats were fasted overnight prior to the incubation studies. Even in these studies addition of glycerol to the buffer did not increase the rate of fatty acid synthesis.

In rat epididymal adipose tissue, the in vitro rate of fatty acid synthesis was much lower when glycerol was added to the buffer than observed in the glucose-containing buffer. This observation is in agreement with results obtained by Persico et al. (43) and Herrera et al. (44) as well as with the observation that rat adipose tissue has much lower glycerokinase activity than does rat liver.

Glycerol is metabolized in mammals primarily by the liver (35). It is possible that the metabolism of glycerol within the tissue might regulate glycerol uptake (34, 35). Glycerol kinase is involved in the first step of glycerol metabolism in liver, therefore, factors that could modify the catalytic activity of this enzyme would be important in the regulation of glycerol utilization and subsequent influence of glycerol on metabolic processes. The activity of this enzyme is a function of the ATP concentration and is also dependent on the concentration of the second substrate-glycerol (39). Glycerol kinase is

inhibited by ADP, AMP and L-α-glycerophosphate (38, 39). High rates of glycerol phosphorylation might deplete ATP levels but increase ADP, AMP and L-α-glycerophosphate in vitro. A decrease in ATP content should decrease the ATP-dependent conversion of acetate to fatty acids when glycerol is present in the incubation medium. However, in the present study addition of glycerol and acetate or pyruvate to the buffer did not decrease the rate of fatty acid synthesis. These results suggest that glycerol might affect hepatic fatty acid synthesis by inhibiting glucose conversion to pyruvate. Other studies (74, 75) have reported that glucose was the main product of glycerol metabolism by intact isolated rat-liver cells. It appears that trioses formed from glycerol are directed toward glucose formation and that glycerol or a metabolic product of glycerol actually depresses the rate of glycolysis in rat liver slices.

Glycerol inhibited glucose but not pyruvate or acetate conversion to fatty acids in rat liver slices. In this regard, the effect of glycerol is similar to the effect of 1,3-butanediol observed in the rat (76). Addition of 1,3-butanediol to the buffer depressed glucose conversion to fatty acids but did not affect acetate conversion to fatty acids in rat liver slices (76). In this report, they postulated that the decrease in hepatic fatty acid synthesis might result from an increased cytoplasmic

$NADH/NAD^+$ ratio following 1,3-butanediol metabolism. α-Glycerophosphate conversion to dihydroxyacetone phosphate in the liver results in the subsequent conversion of NAD^+ to NADH. Thus, the decrease in hepatic fatty acid synthesis observed in rat liver slices might result from an increase in the cytoplasmic $NADH/NAD^+$ ratio following glycerol metabolism. Gumaa et al. (77) demonstrated that the rate of hepatic fatty acid synthesis has an inverse relationship with the cytoplasmic $NADH/NAD^+$ ratio. Recently, Cryer et al. (5) have reported that the $NADH/NAD^+$ ratio was markedly increased in the hepatic cytoplasm after feeding glycerol to rats for 14 days.

The influence of glycerol on fatty acid synthesis in chicken liver slices was also examined. Addition of glycerol to the buffer did not significantly alter the rates of fatty acid synthesis in chicken liver slices (Table 4). This is in contrast to the observations with rat liver slices where a dramatic depression in the rate of fatty acid synthesis was observed when glycerol was added to the buffer. The observed differential response in fatty acid synthesis from glycerol in rat liver slices and chicken liver slices might possibly be explained by a relatively impaired uptake of glycerol or by low activity of glycerokinase in chicken liver. However, glycerol is a good substrate for gluconeogenesis in the chicken (78)

and the reported activity of glycerokinase in chicken liver is as high as that observed in rat liver (48, 57).

The effect of glycerol is similar to the effect of 1,3-butanediol on fatty acid synthesis in chicken liver slices (79). Since steps of glycerol metabolism are similar to 1,3-butanediol metabolism, the similar mechanism might be involved in influencing fatty acid synthesis following glycerol metabolism. The metabolic conversion of 1,3-butanediol to β-hydroxybutyrate shifts the cytoplasmic redox state in rat liver. This increase in NADH/NAD^+ ratio has been postulated to affect fatty acid synthesis in rat liver. However, the redox state was decreased when chicks were fed the 1,3-butanediol-containing diets (79). Evidence for how the redox state in chicken liver is altered by glycerol metabolism is still lacking.

Responses to dietary glycerol have been studied in rats (3, 6, 27, 28, 29, 31), but only few have been reported in chickens (25). There is no a priori reason to assume that chickens would respond to such nutritional manipulation in a manner similar or dissimilar to that of rats. Feeding diets containing 20-parts glycerol to rats or chickens did not influence the growth rate of the animals. However, replacement of glucose with 40-parts glycerol did significantly depress growth rate in both rats and chicks. These animals consumed less food than did the glucose-fed animals.

The findings of increased activities of fatty acid synthetase, malic enzyme and citrate cleavage enzyme in livers of rats fed the glycerol-containing diets are in agreement with reported observations (3, 6). Generally when the activities of these hepatic lipogenic enzymes are elevated the rate of fatty acid synthesis is also increased. However, the hepatic lipogenic enzyme activities were increased without a concomitant increase in hepatic fatty acid synthesis in rats fed glycerol-containing diets.

In general when a substance enters the pathway at some point, the metabolites of this substance may influence the enzyme activity below the point at which the substance enters. Animals do exhibit a degree of flexibility in controlling the quantity of hepatic enzymes to cope with the influx of dietary substrate (1, 2, 3, 4, 5, 6, 7). Glycerol is metabolized very rapidly (80) and thus results in a greater production of α-glycerophosphate than does the metabolism of glucose (5, 24, 26). This α-glycerophosphate can be subsequently converted to dihydroxyacetone phosphate. Dihydroxyacetone has been postulated to be an inducing agent for liver-type pyruvate kinase (81). If availability of glycerol or of a metabolic product of glycerol metabolism can act as an inducer of the enzymes of fatty acid synthesis, it might explain the elevated enzyme activities in rats fed glycerol as compared to glucose. But the impaired rate of fatty acid

synthesis in glycerol-fed rat liver raises the possibility that fatty acid synthesis may not be simply a matter of alterations of lipogenic enzyme activities. Another alternative possibility is that glycerol feeding stimulates the lipogenic enzyme activities but somehow blocks the pathway of fatty acid synthesis. Consistent with this hypothesis is the finding that liver slices incubated with glycerol exhibit a depressed rate of fatty acid synthesis in the rat (Table 2).

As described previously Cryer et al. (5) have demonstrated that rats fed glycerol exhibit an increase in cytoplasmic NADH/NAD^+ ratio but a decrease in citrate concentration resulting from hepatic glycerol metabolism. This increase in the cytoplasmic NADH/NAD^+ ratio might be involved in the reduction of fatty acid synthesis in rat liver (77). I postulate that the depression in hepatic fatty acid synthesis observed when rats were fed glycerol-containing diets, might also be related in part to a reduction of citrate. It has been reported that citrate is a substrate for fatty acid synthesis in the fed rat (82) and citrate is also an activator of acetyl-CoA carboxylase (83). However, delineation of the effects of dietary glycerol on lipogenesis in the rat requires further investigations.

The liver is the major lipogenic organ in the chicken (2), however, the influence of glycerol on

lipogenic enzyme activities and on the rate of fatty acid synthesis in this species has not been studied. Unlike the observations in the rats, the activities of fatty acid synthetase, citrate cleavage enzyme and malic enzyme were not increased in chickens fed glycerol-containing diets for 3 days (Table 7). In the 3-week study both the activities of the lipogenic enzymes and the rate of fatty acid synthesis were substantially depressed in the chicken. Failure to observe an increase in lipogenic enzyme activities in chickens fed glycerol-containing diets suggested that glycerol feeding has a species-specific response, indicating that enzymes which respond to a certain dietary manipulation in one species may not respond in others.

In the 3-day study the activity of fatty acid synthetase was not changed but the rate of fatty acid synthesis was depressed when chickens were fed the glycerol-containing diets. However, in the 3-week study both the activity of fatty acid synthetase and the rate of fatty acid synthesis were depressed. This suggests that the short term control of fatty acid synthesis lies before the fatty acid synthetase step in the chicken. It seems reasonable that the decrease in lipogenic enzyme activities observed when chickens were fed glycerol-containing diets was associated with a significant depression in the rate of fatty acid synthesis.

Despite the emphasis on liver, the adipose tissue is also an important site for fatty acid synthesis in the rat (1). Unchanged lipogenic enzyme activities in adipose tissue was observed when rats were fed glycerol-containing diets for either 3 days or 3 weeks (Table 9). The rates of adipose fatty acid synthesis were also similar in rats fed 20-parts glycerol-containing diets or high-glucose diets (Table 9). Possibly the glycerol consumed by the rat was metabolized mainly in liver and largely converted to glucose. The blood glucose levels of rats did not change significantly after glycerol feeding (6). It appears that in the glycerol-fed rat the circulating glucose was taken up by the adipose tissue and converted to fatty acids at a rate similar to that observed in rats fed high-glucose diets.

Based on the in vitro and in vivo rates of fatty acid synthesis data, it appears that the relative importance of the liver to total de novo fatty acid synthesis did not increase while that of the adipose tissue remained unchanged when rats were fed glycerol rather than glucose diet. Whereas the rates of fatty acid synthesis were depressed when chickens were fed glycerol-containing diets. The differential responses to glycerol feeding between rats and chickens suggested these two animal models have different mechanisms for controlling metabolism in certain aspects and point out the necessity for further

investigation of differences between these species. Results from these experiments indicate that there are species-specific as well as organ-specific metabolic responses to various dietary carbohydrates. Since liver is the major site of fatty acid synthesis in man (11, 12) as well as in chicken (2), further investigations of the influence of glycerol on hepatic lipogenesis and its regulation in the chicken may contribute significantly to our better understanding of the regulation of lipid metabolism in man.

SUMMARY

Apparently, there are tissue-specific as well as species-specific responses to glycerol feeding. The remarkable increase in the lipogenic enzyme activities in livers of rats fed the glycerol-containing diets are in agreement with reported observations (3, 6). However, the hepatic lipogenic enzyme activities were increased without a corresponding increase in the rate of fatty acid synthesis in the rat. Usually, when the activity of an enzyme, as measured in vitro, changes significantly in a given direction a concomitant change generally has occurred in that particular portion of the pathway in which the enzyme participates. However, this did not occur in livers of rats fed glycerol. Thus in addition to an alteration of enzyme pattern, as determined in vitro, there are other factors also regulating in vivo fatty acid synthesis. Since the initial steps of glycerol metabolism are similar to 1,3-butanediol metabolism, it is possible that similar mechanisms might be involved in regulation fatty acid synthesis when these two compounds are fed.

Recently, Cryer et al. (5) presented convincing evidence showing that rats fed glycerol exhibit an increase

in the cytoplasmic NADH/NAD^+ ratio but a decrease in citrate concentration. The cytoplasmic NADH/NAD^+ ratio is also increased when rats were fed 1,3-butanediol. These metabolic shifts might be involved in the control of fatty acid synthesis in rat liver. In contrast to the rat, the activities of lipogenic enzymes were not increased in chickens fed glycerol-containing diets. In fact, it seems reasonable to explain the decrease in lipogenic enzyme activities as reflecting alterations in metabolic activity of the pathway in the chicken fed glycerol-containing diet.

The differential responses to glycerol feeding between rats and chickens point out that different mechanisms for controlling lipid metabolism in these two animal models do exist. The discrete rate-limiting steps and how these steps are influenced by dietary substrate influx requires further investigation.

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