in galactose spot, showing important nucleotide hydrolysis. When UN is incubated with sera, hydrolytic activities of these fluids are inhibited, the importance of galactose 1-P amount being that found in UN only, about 6% of total radioactivity.

These inhibitory processes are less important in assays containing UA; in fact, the ratio of galactose is enhanced in incubation mixtures with sera from ascitic mice. In these mixtures, a fraction of radioactivity is recovered as a new spot migrating faster than galactose. This latter is now being identified.

With further transfer studies those concerning nucleotide sugar hydrolysis in urines and sera from normal and ascitic mice are now being undertaken.

Regulation of Liver Lipogenic Enzymes by Dietary Fats

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Summary. The hepatic lipogenic enzyme levels are more in rats on a fatfree diet and less in unsaturated fat-fed rats, the saturated fat-fed ones remaining in between.

There have been many reports of nutritional and hormonal effects on enzyme systems associated with the metabolism of lipids[1–4]. For some time, our laboratory has been interested in studying various factors controlling lipid metabolism, and the present paper summarizes our studies so far on the effects of dietary fats on some of the liver enzymes involved in fat metabolism. The enzymes studied are α-glycerol *p*-dehydrogenase, glycerol kinase, citrate cleavage enzyme, malic enzyme, malate dehydrogenase and isocitrate dehydrogenase.

Materials and methods. 40 weanling male albino rats were divided into 5 groups of 8 each and were fed ad libitum. The composition of the diet is shown in Table I. The feeding experiment was conducted for a period of 2 months, after which the animals were killed by decapitation and the liver enzymes estimated. The procedures employed were essentially those of BALDWIN et al.[5]. Liver sample was homogenized in 9 volumes of ice-cold 0.14 *N* potassium chloride solution in a Potter-Elvehjem type homogenizer. This was centrifuged at 0–4 °C for 30 min at 27,000 *g*. The supernatant was removed and used as the enzyme source for all the assays. All the enzyme measurements were carried out in a Carl-Zeiss Spectrophotometer PM Q II Model using quartz cell of 1 cm light path by standard procedures[6].

Results and discussion. The results of the various enzyme levels are shown in Table II. The activity of α-glycerol *p*-dehydrogenase in the safflower oil group of rats is lower than the other group of rats; the fat-free and the fat-free plus cholesterol group show the maximum activity. The glycerol kinase activity seems to be not much affected by the different dietary treatments, although the values for the last 2 groups are slightly higher than the rest. The changes in the activities of these 2 enzymes, although not very significant, appear to represent an increased capacity of the liver for triglyceride synthesis in the fat-free groups. Even the nature of the dietary fats affects the levels of these 2 enzymes at least to a certain extent. This could be a physiological necessity so that the synthesis of fatty acid takes place in the presence of a dietary deficiency of the same. BALDWIN et al.[5], however, showed that the changes in the activities of α-glycerol *p*-dehydrogenase and glycerol kinase took place only in the guinea-piglets and not in the rats.

Table I. Diet composition (%)

Fat source	Fat-free casein	Fat	Corn starch.	Cane sugar	Salt mixture	Vitaminized starch dextrose
Safflower oil	15	30	39	10	4	2
Coconut oil	15	30	39	10	4	2
Completely hydrogenated vegetable fat	15	30	39	10	4	2
Fat free	15	..	60	19	4	2
Fat free + 1% cholesterol	15	..	59	19	4	2

The activities of the citrate cleavage enzyme and the malic enzyme are very much affected by the dietary fat. Our results are in agreement with those reported by BALDWIN et al.[5] who showed that the activities of the citrate cleavage enzyme and the malic enzyme were 7.0 and 9.1 times greater with the fat-free diet than with the high fat diet (15.1% lard). ABRAHAM et al.[1] also noted that the citrate cleavage enzyme was dependent on the nutritional status of the animal. Our results, in addition to supporting the above views, also point out that the amount of unsaturated fat in the diet helps to regulate these enzyme levels, the rats fed unsaturated fat showing a lower level than the coconut or the hydrogenated vegetable oil groups. Formation of acetyl Co-A from citrate is the first step in the extramitochondrial fatty acid synthesis, and therefore an increase in the level of citrate cleavage enzyme obtained in fat-free group of rats shows that the fatty acid synthesis is more in these rats. KORNACKER and LÖWENSTEIN[7] suggested that the citrate cleavage reaction could be the rate controlling reaction with respect to the fatty acid synthesis in vivo. YOUNG, SHRAGO and LARDY[8] showed that the increase in malic enzyme when lipogenesis is high represents a mechanism for retrieval of the large amounts of oxalacetate fragments generated by the citrate cleavage enzyme. Both PANDE et al.[9] and WISE and BALL[10] suggested that some of the NADPH required for fatty acid synthesis may be generated by the malic enzyme-catalyzed reaction.

The malate and isocitrate dehydrogenase activities also are less in safflower oil group compared with the others. The higher dehydrogenase activities of the mitochondria as noted here, as well as the higher swelling rates of liver mitochondria from rats fed a diet deficient in EFAs[11],

Table II. Effect of dietary fats on liver enzymes

Enzyme	Diets				
	Safflower oil	Coconut oil	Hydrogenated vegetable fat	Fat-free	Fat-free with cholesterol
α-glycerol *p*-dehydrogenase (E.C.1.1.1.8)	8.9±1.5	10.1±2.1	9.2±1.9	13.4±2.5	14.5±2.7
Glycerol kinase (E.C.2.7.1.30)	0.85±0.15	0.9±0.15	0.9±0.19	1.2±0.1	1.2±0.12
Citrate cleavage enzyme (E.C.4.1.3.7)	2.25±0.20	5.7±0.20	5.3±0.35	8.5±1.2	10.1±1.2
Malic enzyme (E.C.1.1.1.38)	3.4±1.2	10.75±1.6	10.55±2.25	17.6±4.56	18.9±4.5
Malate dehydrogenase (E.C.1.1.1.37)	25.5±4.7	27.9±4.7	27.0±5.2	28.5±3.8	28.5±4.5
Isocitrate dehydrogenase (E.C.1.1.1.42)	24.5±3.8	25.7±3.7	26.5±2.9	28.9±4.9	29.5±4.7

The above values are units of the enzyme/g liver. 1 unit of the enzyme in all the cases is taken as the amount which causes an optical density change of 0.001 per min in the system. The figures are the mean of 8 values along with SD.

provide supporting evidence for the hypothesis that EFA deficiency causes alterations in certain structures of the animal tissues[12]. Previous work from this laboratory (unpublished data) revealed that high amounts of dietary saturated fat caused a marked decrease in PUFA levels in the mitochondria, especially in the mitochondrial phospholipids, and the structural alterations were probably caused by the lack of PUFA in the tissue. These in turn might be responsible for the differences noted in the mitochondrial enzyme levels.

The general nature of the enzymatic changes noted here are consistent with the previous reports which indicated that as the fat content of the diet decreased, rates of fatty acid synthesis and the enzyme activities involved in these functions increased. In addition, our results also show that the activities of many of these enzymes are altered as a function of unsaturated fat intake. The differences in the liver fatty acid profile due to the feeding of different fats to rats, when accompanied by the changes in the enzymatic levels noted here, are in support of the observations made by ALLMAN et al.[3].

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ESR-Spectroscopic Changes on Enzymatic Depolymerization of Spin-Labelled Amylose

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Summary. The ESR-spectra of nitroxyl-labelled amyloses (molecular weight 14,000) show anisotropic effects. Depolymerization with α-amylase alters the spectra, and the rate of change is dependent on concentration of enzyme.

When a nitroxyl group is introduced into a macromolecule, the ESR spectrum of the spin-labelled compound in solution reflects its molecular size. The relatively slow tumbling of the large molecule causes imperfect averaging of anisotropic magnetic interactions, and the resulting spectrum differs from that observed for a small nitroxide. The affect has been applied in the study of proteins[1] and synthetic polymers[2]. The expectation that such effects would be observed also for spin-labelled amylose formed the basis of the present work[3]. Since the ESR-spectrum would depend on molecular size, it should furthermore be sensitive to depolymerization. The action of amylase could therefore be recorded as a continuously changing spectrum. Synthetic dyes have been linked to amylose substrates for the purpose of assaying amylase[4–6]. These methods, however, all involve sampling, whereas the use of a spin-labelled substrate offers the prospect of

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