cosal scrapings, homogenates, and mitochondria from normal rats and guinea pigs, and mucosal scrapings and everted intestinal segments from bile-fistula rats, failed to oxidize cholesterol-26-^{14}C to $^{14}CO_2$. It is concluded that oxidation of side-chain in the intestinal mucosal cell is not a quantitatively significant metabolic fate of cholesterol.

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Alterations in Serine Metabolism in Rats Fed Liquid Synthetic Diets.* (31493)

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The liquid synthetic diet proposed by Greenstein *et al,* has been found to support good growth rates, reproduction and lactation in rats(1,2). Therefore, this diet has been considered nutritionally complete and the content of essential plus non-essential amino acids considered optimal. This diet has recently been used as a control diet in studies of hepatic serine biosynthesis in rats. Prompt alterations in the concentration of several enzymes important in serine metabolism were noted. This observation suggests that synthetic diets supporting normal or near normal growth rates may nevertheless require significant compensatory changes in enzyme levels to provide required metabolites. The importance of this observation to the study of alterations caused by specific dietary deficiencies is apparent. These studies also demonstrate the importance of dietary amino acids in the regulation of enzyme levels in serine metabolism.

Methods. Male Osborne-Mendel rats (40-80 g) were used in these studies. Liquid synthetic diets, based on the diet of Greenstein *et al*(2), (Diet A) were prepared by General Biochemicals, Inc. (Diet No. 116). Identical diets were also prepared omitting serine and glycine (Diet B) or serine, glycine and cysteine (Diet C). Rats were pair fed these liquid diets from Richter feeding tubes. Control rats were fed, *ad libitum,* either Purina Lab Chow or a solid 25% casein diet(3). The rats were fed the diets for 7 days, killed and enzyme preparations made from liver as described previously(3). Assays for 3-P-glycerate† dehydrogenase, using 3-P-glycerate as substrate, serine dehydratase and D-glycerate dehydrogenase have been described(3). One unit of enzyme activity is defined as a change

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† 3-P-glycerate = 3-phosphoglycerate.

TABLE I. Comparison of Enzyme Levels in Liver of Rats Fed Chow or Liquid Synthetic Diets.

Diet*	No. animals†	3-P-glycerate dehydrogenase‡	Serine dehydratase‡	D-glycerate dehydrogenase‡
		Units/mg protein ± S.D.		
Chow	10	3.1 ± 1.4	119.8 ± 35.7	25.7 ± 4.5
A	10	7.1 ± 2.2	51.5 ± 38.6	37.6 ± 6.5
B	10	11.8 ± 3.1	7.0 ± 3.0	33.3 ± 7.5
C	7	9.4 ± 2.2	10.7 ± 4.1	—

* Diet A—complete liquid synthetic diet; Diet B—liquid synthetic diet omitting serine and glycine; Diet C—liquid synthetic diet omitting serine, glycine, and cysteine.

† Rat weight 65-80 g.

‡ 3-P-glycerate dehydrogenase and serine dehydratase were statistically different ($p < .01$) for groups chow, A and B. Rats fed diet C were not different from B but were ($p < .01$) from chow and A. No significant differences were noted for D-glycerate dehydrogenase.

in absorbency at 340 mμ of 0.001 per minute. All materials were obtained from prior sources (3).

Results. Weight gain in rats fed chow, 25% casein or diets A, B and C were equivalent for a 7-day period. However, an increase in hepatic 3-P-glycerate dehydrogenase concentration was noted when rats were fed the synthetic liquid diet (A), (Table I). This increase was significant ($p < .01$) when compared to chow fed rats and was accompanied by a marked decline in serine dehydratase levels. Enzyme levels for rats fed chow and 25% casein diets were virtually identical and data are shown for the chow fed group. The small increase in D-glycerate dehydrogenase in rats fed diets A or B was not significant ($p > .05$). The observed changes in 3-P-glycerate dehydrogenase and serine dehydratase were not prevented by doubling the serine and glycine content of the liquid synthetic diet (Table II).

A further increase in 3-P-glycerate dehydrogenase occurred when either serine and glycine (Diet B) or serine, glycine and cysteine (Diet C) were removed from the liquid synthetic diet, (Table I). Similar results have been obtained in 6 separate experiments using rats of various ages.

TABLE II. The Effect of Added Serine and Glycine to the Liquid Synthetic Diet.

Diet*	3-P-glycerate dehydrogenase†	Serine dehydratase†
	Units/mg protein ± S.D.	
Chow	3.5 ± 1.4	96.3 ± 44.3
Diet A plus serine and glycine	9.1 ± 2.6	23.1 ± 4.9

* Diet A plus 7.7 g L-serine and 15 g glycine added per liter.

† Differences statistically significant ($p < .01$).

The dietary induced changes in enzyme concentrations have been confirmed and explored more completely by studies using solid synthetic diets of variable protein content(3). These latter studies demonstrated a rise in 3-P-glycerate dehydrogenase and P-serine phosphatase and a fall in serine dehydratase levels in rats fed protein deficient diets (2% casein). These effects on 3-P-glycerate dehydrogenase and P-serine phosphatase were largely reversed by addition of 1% cysteine (by weight) to the deficient diet. Thus, the enzyme changes observed in rats fed apparently adequate liquid synthetic diets closely resemble those produced by protein deficient diets. The effect of cysteine on these latter changes suggested that a relative cysteine deficiency might, at least in part, account for the altered enzyme levels in the rats fed liquid diets. This is supported by evidence that the removal of cysteine from the synthetic diet(A) results in increased levels of 3-P-glycerate dehydrogenase similar to those observed when serine and glycine were removed from the diet. Since serine is a direct precursor of cysteine in mammals(5) such an effect might be anticipated.

Discussion. Previous, careful studies have demonstrated the ability of several chemically defined liquid synthetic diets to support normal growth rates in rats(1,2). Such diets have been considered "nutritionally complete" (2). However the present studies clearly demonstrate that significant alterations in the metabolism of amino acids may occur despite

normal growth rates, reproduction and longevity in rats(1). It seems likely that the changes in hepatic enzyme concentration noted in rats fed the synthetic liquid diets may be associated with increased endogenous synthesis of serine from carbohydrate sources. Therefore, normal growth rates may be achieved and sustained with marginal diets if considerable reserve capacity exists for the endogenous synthesis of necessary metabolites. The concentration of other biosynthetic enzymes and intermediary substrates may also vary considerably when synthetic amino acid diets adequate to maintain growth are substituted for protein containing diets. The effects of such refined diets on intermediary metabolism must be carefully defined and compared with diets of known protein content before interpretations are made on the effects of manipulating various constituents of the synthetic mixture(4).

These studies have further confirmed the responsiveness of the "phosphorylated" pathway of serine biosynthesis to dietary factors. Addition of serine and glycine to the liquid synthetic diet did not prevent the rise in enzyme levels associated with this diet. However, removal of serine and glycine from the liquid synthetic diet resulted in a further increase in 3-P-glycerate dehydrogenase level. This latter effect may result from further restriction of total amino acid intake or from a secondary deficiency of cysteine, an ultimate end product of serine metabolism. This latter interpretation is supported by data suggesting the importance of dietary cysteine in regulating enzyme concentration in the "phosphorylated" pathway of serine biosynthesis (3).

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Lysozymuria Induced in Rats by Nephrotoxic Agents. (31494)

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Lysozymuria has been found in various renal diseases of man and has also been demonstrated after administration of mercuric chloride to rats(1,2). It has been suggested that measurement of this enzyme may become an important tool in the diagnosis of renal disease.

Since the pathogenesis of lysozymuria has not been established, experiments were conducted to investigate this problem in toxic tubular nephroses in rats.

Materials and methods. Male, albino rats of Sherman (Lederle) and Wistar (Royal Hart), strains, weighing 300-400 g, were used. Single doses of sodium chromate (Na_2CrO_4) subcutaneously, mercuric chloride ($HgCl_2$) and uranyl nitrate ($UO_2(NO_3)_2$) intraperitoneally were administered. The rats were placed in metabolism cages. Urine was collected in containers surrounded by dry ice and the volume was recorded. Concentrations of protein and glucose were estimated by a reagent strip (Combistix®)* and specific gravity by a Goldberg type refractometer.† Food and water were withheld for various periods to permit measurement of renal concentrating ability. Urine and kidney glutamic oxalacetic acid transaminase (GOT) was determined by the Sigma Frankel method(3). Urine, plasma and tissue lysozymes were assayed as described by Prockop and Davidson(2). For the determination of

* Ames Co., Elkhart, Ind.
† American Optical Co., Buffalo, N. Y.