muscle extracts were assayed with or without $HgCl_2$-GSH reagent (Table II).

Two of the G-6-PDH preparations had significant hexokinase activity, which poses a problem, since glucose and ATP are present in most tissue extracts. However, when $MgCl_2$ was omitted and EDTA added, hexokinase was completely inhibited without appreciable loss of G-6-PDH activity(4).

To test recovery of G-6-P, a measured amount was added to a $HClO_4$ extract of muscle prior to neutralization; a 70% increase in G-6-P content was observed in quadruplicate determinations, and this agreed within 1% with the theoretical value.

Discussion. Steiner and Williams(5) recently showed that GSSG in liver extracts interferes with enzymatic assay of G-6-P. They were able to remove the GSSG with Dowex 50. G-6-P was separated from free glucose in their extracts by precipitation with barium and alcohol. The present procedure makes these additional steps in determination of G-6-P unnecessary.

The important but separate problem of obtaining tissue extracts without stimulating glycogenolysis has not been considered in this presentation.

Summary. A method is described for measurement of glucose-6-phosphate in tissue extracts by use of glucose-6-phosphate dehydrogenase. Interference resulting from contamination of this enzyme with glutathione reductase was abolished by addition of a mixture of $HgCl_2$ and glutathione. Hexokinase, another contaminating enzyme which interfered with the determination, was inhibited by removing Mg ion. Phosphoglucomutase and phosphoglucoseisomerase were not present in significant amounts.

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Regulation of Cholesterol Mobilization in Mice by Bile Acids.* (25584)

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In a study of effects of several bile acids on cholesterol levels and synthesis rates in mice (1), 2 of these substances—cholic and hyodeoxycholic acids—had definite but opposing effects. Cholic acid caused substantial increase in liver cholesterol levels, which triggered 50% decrease in rate of acetate-1-C^{14} incorporation into liver total cholesterol. On the other hand, hyodeoxycholic acid decreased liver cholesterol, and greatly increased synthesis rate. Since previous experiments(2,3) had shown that cholic acid greatly retards mobilization of accumulated mouse liver cholesterol, it seemed of interest to test the effect of hyodeoxycholic acid on the same parameter to see if it would effectively increase the rate of cholesterol mobilization.

Procedures. Two hundred twenty Webster strain female, albino mice, weighing 18-20 g, were placed on a synthetic basal cholesterol free diet (CFD)(4) for 2 weeks, and then divided into 2 groups. Fifty mice received unsupplemented basal diet (CFD), while remaining 170 mice were placed on diet of CFD plus 0.5% cholic acid and 1% cholesterol. At end of 3-week cholesterol build-up, 10 normal mice and 10 mice from the build-up group were sacrificed for liver and carcass choles-

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TABLE I. Mobilization of Accumulated Liver Cholesterol in Mice Fed Cholic and Hyodeoxycholic Acids, as a Function of Time.

Group	Liver total cholesterol, mg/g liver				
	0 time	1 wk	2 wk	3 wk	4 wk
A. Normal—CFD*	6.59 ± 1.56	6.74 ± 1.61	6.84 ± 1.11	7.02 ± 1.56	7.93 ± 1.90
B. Regression—CFD	18.7 ± 4.06	20.3 ± 5.40	15.3 ± 2.90	10.5 ± 2.45	7.67 ± 2.17
C. *Idem* + 0.5% cholic acid	18.7 ± 4.06	19.4 ± 3.03	19.0 ± 3.54	18.4 ± 3.70	18.1 ± 2.60
D. " + 0.5% hyodeoxycholic acid	18.7 ± 4.06	8.46 ± 6.17	3.28 ± 1.05	3.21 ± .51	3.57 ± .93
E. " + 0.5% cholic acid + 0.5% hyodeoxycholic acid	18.7 ± 4.06	9.56 ± 5.23	6.21 ± 2.12	6.36 ± 2.30	3.98 ± .55

Statistical comparison of values:

Groups	1 wk	2 wk	3 wk	4 wk
A and B	$P < .01$	$P < .01$	$P < .01$	$P = .76$
A and C	"	"	"	$P < .01$
A and D	$P = .52$	"	"	"
C and E	$P < .01$	"	"	"

* CFD = cholesterol-free diet. ± numbers are stand. dev.

terol determination. The remaining 40 normal (Group A) mice were continued on CFD, while mice with elevated cholesterol were divided into 4 regression groups of 40 mice each: Group B, regression controls, were returned to CFD; Group C received CFD plus 0.5% cholic acid; Group D, CFD plus 0.5% hyodeoxycholic acid; and Group E, CFD plus 0.5% cholic acid and 0.5% hyodeoxycholic acid. At weekly intervals one-fourth of mice from each group were sacrificed, their livers removed and analyzed for total cholesterol(3). Stomachs and intestines were removed and cleared of all ingested material, then hydrolyzed together with the carcass and blood by refluxing for 3 hours in 5% alcoholic potassium hydroxide. Aliquots of the hydrolysate were used for total cholesterol determination (3).

Results. Several interesting findings resulted. Regression (Table I) of accumulated (187% increase) liver cholesterol of Group B mice required a total of 4 weeks after return to the cholesterol free diet (CFD). On the other hand, animals fed CFD plus 0.5% cholic acid (Group C) showed no regression of elevated liver cholesterol within the same interval. The accumulated liver cholesterol of animals fed CFD supplemented with 0.5% hyodeoxycholic acid (Group D) regressed to near normal levels by end of first week, and dropped to 50% below normal cholesterol level by the end of 2nd week. This level was maintained through the end of 4th week. Addition of equal percentages of both bile acids (Group E) showed that at this concentration hyodeoxycholic acid reverses the inhibitory effect of cholic acid on liver cholesterol mobilization.

Data on mobilization of accumulated mouse carcass cholesterol are presented in Table II. There was a 26% increase in carcass cholesterol during the 3-week build-up period. Regardless of dietary supplement, carcass cholesterol levels returned to normal by the end of 2nd week.

It appears that in the mouse the control of cholesterol metabolism in extrahepatic tissues —with exception of blood(4)—is independent of the effects of bile acid and of liver *per se*.

The mechanisms by which bile acids influence liver cholesterol mobilization remain doubtful. Cholic acid may inhibit cholesterol mobilization (a) by preventing excretion of α and β sterols, or (b) by blocking oxidation of cholesterol to bile acids obligatory for secretion(5,6). Hyodeoxycholic acid may have the opposite effects: that is, it may promote rapid excretion of α and β sterols, or may increase the rate of cholesterol oxidation. The reversal

TABLE II. Mobilization of Accumulated Carcass Cholesterol in Mice Fed Cholic and Hyodeoxycholic Acids, as a Function of Time.

Group	Carcass* total cholesterol, mg/g carcass				
	0 time	1 wk	2 wk	3 wk	4 wk
A. Normal—CFD†	2.45 ± .38	2.43 ± .19	2.66 ± .29	2.55 ± .36	2.55 ± .37
B. Regression—CFD	3.08 ± .43	3.05 ± .16	2.60 ± .19	2.48 ± .29	2.33 ± .49
D. *Idem* + 0.5% hyodeoxycholic acid	3.08 ± .43	3.11 ± .29	2.76 ± .15	2.68 ± .21	2.62 ± .20
E. " + 0.5% cholic acid + 0.5% hyodeoxycholic acid	3.08 ± .43	3.09 ± .14	2.68 ± .19	2.56 ± .37	2.78 ± .35

Statistical comparison of values:

Groups	0 time	1 wk	2 wk	3 wk	4 wk
A and B	$P < .01$	$P < .01$	$P = .64$	$P = .69$	$P = .32$
A and D	"	"	$P = .42$	$P = .45$	$P = .69$
A and E	"	"	$P = .90$	$P \cong 1.00$	$P = .24$

* without liver. † CFD = cholesterol-free diet. ± numbers are stand. dev.

of cholic acid effects by hyodeoxycholic acid suggests that the latter substance acts as an antimetabolite of cholic acid. Although general increases or decreases in metabolic rate would have similar effects on cholesterol mobilization, we have shown(4,7) that under our experimental conditions, the metabolic rate remains constant.

It was hoped that the effects of hyodeoxycholic acid in the mouse could be duplicated in other species, but in preliminary studies in the rat, this substance was ineffective at equivalent dosage levels.

Summary. The effects of cholic and hyodeoxycholic acids on mouse liver and carcass cholesterol mobilization were investigated. Cholic acid inhibited liver cholesterol mobilization, while hyodeoxycholic acid promoted a rapid elimination of accumulated liver cholesterol. Hyodeoxycholic acid reversed the inhibition caused by cholic acid. Neither of these substances effected the rate of carcass cholesterol mobilization.

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Aliphatic Amino Compounds as Inhibitors of Plasminogen Activation. (25585)

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The fibrinolytic system of man is the subject of increasing interest(1). While activators such as streptokinase have been studied extensively, inhibiting agents have received relatively little attention. ε-amino-n-caproic acid (ε-ACA) was first mentioned in this connection in 1957 by Japanese workers who described it as an anti-plasmin agent(2). It was shown subsequently by Ablondi *et al.*(3)

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