is worthy of further investigation.

It is evident from the experiments reported in this paper that a partial deficiency of threonine causes marked changes in the activities of several liver enzymes as well as in the extent of liver fat deposition. Since these marked changes occur concurrently, it is suggested that the liver fat accumulation observed in animals fed 9% casein-sucrose diets is a reflection of an alteration in the activity of one or more enzymes important for normal liver function.

Summary. The effect of a partial deficiency of threonine in the rat on the activities of several liver enzymes and on liver fat deposition has been determined. Liver fat deposition, in confirmation of earlier reports, was higher in animals receiving the threonine-deficient diet. The activities of the mitochondrial enzymes, succinic oxidase and choline oxidase, were higher in liver homogenates from threonine-deficient animals. Endogenous respiration and the activities of the cytoplasmic enzymes, xanthine oxidase and tyrosine oxidase, were lower in the livers of animals fed diets deficient in threonine.

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Role of Bile Salts in Activity of Cholesterol Esterase. (20666)

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Nedswedski(1-3) showed that bile salts were required for the synthesis of cholesterol ester from colloidal solutions of cholesterol and higher fatty acids by pancreatic cholesterol esterase. In the absence of bile salts, the enzyme was completely inactivated in 24 hours, but if bile salts were present, it retained its activity almost completely for 10 days. It was postulated that the bile salts exert their effect by stabilizing the enzyme. Klein(4) observed that hydrolytic cholesterol esterase of cattle pancreas was activated by bile acids, especially cholic acid. Nomura(5), Sym(6), and Vercellone(7) reported similar observations. Recent reports(8,9) have presented improved procedures for studying hydrolytic and esterifying cholesterol esterase systems. Both systems were demonstrated in pancreas(8,9) dog serum(10), and rat intestinal mucosa(11). The enzyme or enzymes occurring in these three tissues were alike in respect to optimum pH, inactivation temperature, and in requiring bile salts for activity. Since the substrate emulsions employed in our experiments(8-12) were stable and uniform in composition in absence of bile salts, it

seemed unlikely that the effect of bile salts on activity of cholesterol esterase was due to their emulsifying action on the substrates. Also, we observed repeatedly that pancreatic homogenates, stored at 5-7°, retain full activity for 20-30 days in the absence of bile salts. Our experiments have confirmed previously reported requirement of bile salts for cholesterol esterase activity, yet the results have not been in accord with any of the suggested explanations.

In view of this and the possible role of this enzyme in absorption of cholesterol(13) it seemed desirable to investigate further the *mechanism* of the effect of bile salts on cholesterol esterase activity. First, the comparative effect of different bile salts was determined in both the hydrolytic and esterifying cholesterol esterase systems. Of the bile salts tested, sodium taurocholate and cholate were most active. It was observed that bile acids having 3, 2, and 1 hydroxyl groups respectively showed a decreasing order of activity while sodium dehydrocholate with no hydroxyl groups was inactive in promoting either synthesis or hydrolysis. Also evidence was obtained that optimal cholesterol esterase activity occurs when bile salts and cholesterol are present in a ratio of 1:1; an excess of the former produced inhibition of esterifying activity.

Methods and materials. Fresh frozen hog pancreas was used as enzyme source and the homogenates prepared as previously described (11). The substrate mixtures were prepared as described earlier(8,9) and contained cholesterol and oleic acid in experiments on the esterifying cholesterol esterase system and cholesterol oleate in those on the hydrolytic system. The pH of the digests for hydrolysis was 6.5 and for esterification, 6.1. The different bile salts, (amounts indicated in Table I) were added during preparation of substrate mixtures. One cc of pancreas homogenate equivalent to .2 g fresh tissue was added to 12 cc of substrate mixture in all cases. Incubation temperature was 37°C. In experiments on the esterifying system, samples were removed from the enzyme digests (substrate mixture plus pancreas homogenate) at 0 and either 1 or 3 hours; with the hydrolytic system samples were taken at 0 and 4 hours.

TABLE I. Effect of Bile Salts on Cholesterol Esterase Activity.

Exp.	Bile salt: Type	Bile salt: Conc. (mMol)	Activity*: Esterification† (mMol)	Activity*: Hydrolysis‡ (mMol)
1	Taurocholate	.186	.477	.089
	Glycocholate	"	.076	.044
	Cholate	"	.300	.103
	Desoxycholate	"	.031	.043
	Lithocholate	"	.009	.006
	Dehydrocholate	"	.000	.000
2	Taurocholate	"	.493	.079
	Glycocholate	"	.092	.055
	Cholate	"	.293	.093
	Cholate + taurine	"	.293	.095
	Cholate + glycine	"	.281	.095
3	Taurocholate	.000	.000	.000
	"	.065	.204	.040
	"	.086	.301	.045
	"	.129	.362	.062
	"	.258	.407	.068
	"	.516	.255	.078
	"	.774	.169	.095

* Expressed as mMol cholesterol esterified or liberated by hydrolysis/g fresh tissue/hr.

† Substrate: .258 mMol cholesterol and .774 mMol oleic acid/digest in Exp. 1 and 2. Cholesterol, oleic acid, .258 mMol/digest in Exp. 3.

‡ Substrate: .258 mMol cholesterol oleate/digest.

Other pertinent information given in Table I. Throughout the period of incubation the enzyme was saturated with the substrate so that enzyme digests could be compared with one another in the same experiment. Total and free cholesterol fractions were determined by the method of Schoenheimer and Sperry (14) as modified by Sperry and Webb(15). All calculations based on amounts of free cholesterol present in the digests at zero time and at the end of the incubation period. Results are expressed in terms of mmol of cholesterol esterified or liberated by hydrolysis per g of fresh tissue per hour.

Results. Influence of Different Bile Salts on Cholesterol Esterase Activity. Results shown in Exp. 1, Table I. Sodium taurocholate was the most active bile salt in promoting esterification. In the hydrolytic system taurocholate and cholate had approximately the same activity. Glycocholate was markedly less active in promoting either esterification or hydrolysis than taurocholate or the comparable unconjugated salt, cholate. In the series of unconjugated bile salts a decreasing order of activity was observed with

cholate, desoxycholate, lithocholate, and dehydrocholate. These bile salts have 3, 2, 1, and 0 hydroxyl groups per molecule, respectively.

Effect of Conjugated Versus Unconjugated Bile Salts. The data on the effect of different bile salts indicated that conjugation of cholic acid with glycine markedly decreased the effect of cholate in both esterification and hydrolysis while conjugation with taurine enhanced its effect in esterification. The purpose of Exp. 2 was to ascertain if the conjugated bile salts function as a unit, or whether the components have independent effects. Sodium cholate exhibited the same degree of activation when alone or when in association with equivalent amounts of either taurine or glycine while the corresponding conjugated bile salts, taurocholate and glycocholate exhibited marked differences in promoting synthesis and hydrolysis in comparison to cholate. It appears that the conjugated bile salts function as a unit and that free taurine and glycine have no effect on the activation of the enzyme by cholate.

Effect of Bile Salt Concentration on Cholesterol Esterase Activity. In previous work (9) it was observed that at a cholesterol level of .195 mmol as the amount of bile salt per digest was increased from 0 to .186 mmol there was a marked increase in the level of activity. Above this concentration and up to .280 mmol there were further increases in activity, but of a smaller magnitude. In the previous study the concentration of bile salts did not extend beyond .327 mmol. In Exp. 3 the cholesterol concentration was held constant at .258 mmol and the taurocholate concentration varied from 0 to .774 mmol. As the bile salt concentration was increased up to .258 mmol, the esterifying and hydrolytic activity increased. Above this concentration of bile salts and up to .774 mmol there was a depression of esterifying activity. In the hydrolytic system further activation occurred with increasing concentrations of sodium taurocholate, up to .774 mmol.

Relationship of Substrate to Bile Salt Concentration. Earlier(9) it was shown that increasing the oleic acid concentration from 1 to 2 molar equivalents of cholesterol, with the bile salt concentration constant, doubled the esterifying activity; further increases in oleic acid concentration did not significantly increase the esterifying activity. Bile salt concentration was optimum for synthetic activity at the level of cholesterol used so it seemed that the oleic acid effect was a mass action effect and was independent of the bile salt concentration. This and the observation reported above that the esterifying activity was maximal with the bile salt concentration at a 1:1 ratio with cholesterol suggested that if the bile salt concentration were held constant and the cholesterol (and oleic acid) varied there should be an increase in activity up to a 1:1 ratio of cholesterol to bile salt; higher levels of cholesterol should have no effect. This type of experiment was run with cholesterol and oleic acid at .065, .129, .258, .516, and .774 mmol per digest with the taurocholate held constant at .258 mmol. The cholesterol esterified, as mmol per g fresh tissue per hour, was .153, .296, .430, .423, and .431, respectively. Thus, the esterification was maximal at a molar ratio of 1:1 for cholesterol and bile salt and there was no effect of higher levels of cholesterol.

Conclusions. The data on the different bile salts suggest that several aspects of the structure of the bile salt molecule are involved in their effects on cholesterol esterase activity. Firstly, taurocholic acid has a distinctly greater effect than either glycocholic or the unconjugated cholic acid (the latter in the esterifying system only). Secondly, conjugation with glycine inhibits the effect of cholic acid. Thirdly, free taurine and glycine have no influence on the effect of cholic acid, *i.e.* the effects of taurocholic and glycocholic acids are due to the intact molecules. Fourthly, in the series of unconjugated acids the degree of effect was greatest with cholic acid which has 3 hydroxyl groups in the molecule and the effect decreased with the decreasing number of hydroxyl groups until with dehydrocholic, which has no hydroxyl groups, there was no effect. The results clearly demonstrate that both the hydroxyl groups and the carboxyl group, free or conjugated, are involved in the effect of bile salts on cholesterol esterase activity.

From the previous work, reviewed above, the bile salt effect does not appear to be

related to emulsification of the substrate or the stabilization of the enzyme. The present findings on the interrelationships of cholesterol and bile salt concentration suggest some type of combination between cholesterol and bile salt. The data on the esterification system are especially significant. They indicate that 1 molecule of bile salt is associated with 1 molecule of cholesterol in the reaction with the fatty acid. It seems likely that the levels of activity observed were related to the concentration of a complex between cholesterol and bile salts rather than the concentration of either of the compounds, independently. In the hydrolytic system there was some effect of bile salt concentration above a 1:1 molar ratio with the cholesterol ester. However, the results are in accord with the hypothesis of complex formation between cholesterol or cholesterol ester and bile salt. If the mechanism of the bile salt effect is complex formation, the differences between the different bile salts could be due either to ease of complex formation or a specificity of cholesterol esterase related to the structure of the bile salt molecule.

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Cortisone Effect on Antibody Levels in Rabbits Simultaneously Immunized with Serum Albumin and Sheep Cells.* (20667)

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We have reported(1) that in a rabbit simultaneously immunized with bovine serum albumin and mumps vaccine, treatment with cortisone might markedly suppress the formation of antibody to the serum albumin while leaving the antibody to mumps apparently unaffected as compared with control animals. The antibody to mumps was measured by 3 different serological tests (hemagglutination inhibition, complement fixation and neutralization) which showed good agreement but all 3 tests are at best semi-quantitative and gave no indication of the actual amounts (weights) of the antibody involved. In order to establish whether cortisone might affect 2 different antigen-antibody systems differently in the same rabbit we have investigated the effect of cortisone on the sheep cell hemolysin system in rabbits, this being a system accessible to quantitative study and one in which preliminary experiments indicated no difference whatsoever in the hemolysin or agglutinin serum dilution titers of animals treated or not treated with cortisone.

Materials and methods. Antigens. Rabbits were immunized simultaneously with alum-precipitated bovine serum albumin and with

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