of fine granular lipid material and heavy invasions of large lipid masses in the zona reticularis.

Summary. Large lipid masses were found in zona reticularis of certain porcine animals and it was suggested that they might represent a degenerative change. Correlation coefficients indicated that the zona reticularis lipid masses may be associated with a rapid post-mortem pH decline in muscle and a short post-mortem sarcomere length.

1. Briskey, E. J., Adv. Food Res., 1964, v13, 89.
2. Wismer-Pedersen, J., Briskey, E. J., Nature, 1961, v189, 318.
3. Lawrie, R. A., Gatherum, D. P., Hale, P. P., ibid., 1958, v182, 807.
4. Ludvigsen, J., Acta Endocrinol., 1957, v26, 406.
5. Judge, M. D., Briskey, E. J., Forrest, J. C., Meyer, R. K., in preparation.
6. Sayre, R. N., Briskey, E. J., Hoekstra, W. G., J. Animal Sci., 1963, v22, 1012.
7. Locker, R. H., Food Res., 1960, v25, 304.
8. Meyers, M. W., Charipper, H. A., Anat. Rec., 1956, v124, 1.

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Lipogenesis and Cholesterol Turnover in the Chick as Influenced by Dietary Lithocholic Acid. (30674)

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Dietary lithocholic acid markedly elevates plasma lipids in the chicken(1). The ingestion of this bile acid also results in an increased liver size and a proliferation of bile ductules(1,2). The mechanism(s) by which plasma lipids are elevated as a consequence of lithocholic acid ingestion remains obscure. Elevated plasma lipid levels might result as a consequence of increased synthetic rates and/or a decreased rate of turnover.

The data presented here were obtained in an effort to shed light on the mechanism(s) responsible for the lipemia observed in lithocholic acid-fed chicks.

Materials and methods. Male White Leghorn Hy-line chicks were fed a stock ration prior to being fed the experimental diets. Chicks used in these experiments were fed the lithocholic acid supplemented diet for at least 3 weeks, a period previously shown to be sufficient for the induction of the reported hyperlipemia and ductular hyperplasia(1,2). The diets were identical to those used in previous studies(1) and were fed on an *ad libitum* basis as was water.

The chicks used for *in vitro* studies were sacrificed by cervical fracture and the liver was quickly excised, weighed and slices prepared from the left lateral lobe with a Stadie-Riggs hand microtome. The slices were weighed on a rapid weighing torsion balance and approximately 100 mg of slices were incubated in 25 ml Erlenmeyer flasks having 1.5 × 3 cm glass center wells. The main compartment contained 3.0 ml of Krebs-Ringer bicarbonate buffer pH 7.4. The center wells contained a 2 cm × 2 cm piece of Whatman #1 filter paper and the flasks were capped with rubber plasma stoppers. Incubations were carried out in an atmosphere of 95% O_2-5% CO_2 at 38°C in a metabolic shaker (90 strokes/min). At the completion of the 3-hour incubation period, 0.1 ml of 25% KOH was introduced through the rubber cap with a syringe onto the filter paper and 0.5 ml of 0.2 N H_2SO_4 into the main compartment to insure complete liberation of CO_2 from the medium. Following the incubation, $^{14}CO_2$, fatty acid-^{14}C and cholesterol-^{14}C were determined as previously outlined (3).

Liver used for enzyme assays was homogenized with cold 0.15 M KCl, the homogenate was centrifuged at 1000 × *g* for 15 minutes and the supernatant was used for assay. Glucose-6-phosphate dehydrogenase (G-6-PD) and 6-phosphogluconate dehydrogenase (6-PGD) were measured separately by the meth-

TABLE I. Body Weight Gain, Liver Size and Plasma and Liver Lipids of Chicks Fed a Lithocholic Acid Free or Supplemented Diet.*

	Dietary treatment		
	Control	Lithocholic acid supplemented	Probability of significance
28-day body wt gain, g	510 ± 82†	369 ± 75	<.001
Liver size, g/100 g body wt	2.2 ± .2	7.6 ± 1.4	<.001
Plasma lipids, mg/100 ml			
Total lipids	735 ± 56	2469 ± 491	<.001
" cholesterol	119 ± 7	453 ± 137	<.001
Lipid phosphorus	10.6 ± .6	31.4 ± 7.0	<.001
Liver lipids (wet wt basis)			
Total lipids, %	5.0 ± .6	4.5 ± .6	ns
Cholesterol, mg/g	3.1 ± .3	4.0 ± .4	<.001

* Chicks had an initial weight of approximately 320 g.
† Mean for 9 chicks ± S.D.

TABLE II. Incorporation of Acetate-1-^{14}C and Glucose-U-^{14}C into Fatty Acids and Cholesterol by Liver Slices from Chicks Fed a Lithocholic Acid Free or Supplemented Diet.*

		Dietary treatment		
Addition to buffer	Incorporation into:	Control	Lithocholic acid supplemented	Probability of significance
		(mμmoles incorporated/100 mg tissue/3 hr)		
Acetate-1-^{14}C	Fatty acids	718 ± 315†	1147 ± 409	<.025
	Cholesterol	19.6 ± 7.8	28.2 ± 16.9	ns
Glucose-U-^{14}C	Fatty acids	82 ± 53	229 ± 100	<.005
	Cholesterol	5.8 ± 3.3	9.4 ± 4.4	ns

* 100 mg of slices incubated in 3 ml bicarbonate buffer containing 15 μmoles glucose and 0.3 unit insulin and 0.5 μc of acetate-1-^{14}C or glucose-U-^{14}C for 3 hr at 38°.
† Mean for 9 chicks ± S.D.

od of Horecker and Smyrniotis(4). NADP malic dehydrogenase (malic enzyme) was assayed as described by Ochoa(5). Nitrogen was determined by micro-Kjeldahl digestion followed by nesslerization(6). Enzyme activities are expressed as units/mg N, where a unit is defined as that amount of enzyme which will catalyze the transformation of 1 micromole of substrate per minute at 30°C.

Cholesterol turnover was studied by intravenously administering 7.14 μc of cholesterol-4-^{14}C (specific activity 25.8 μc/μmole) and determining the cholesterol specific activity after 8, 24, 48 and 72 hours. The radioactive cholesterol was administered in an emulsion prepared as described by Meier *et al*(7). Plasma samples were saponified in alcoholic KOH, the cholesterol isolated as the digitonide and radioactivity determined(8).

Plasma and liver lipids were determined as previously described(9). The data were evaluated statistically by means of the t-test.

Results. The effect of dietary lithocholic acid on body weight gain, plasma lipids, liver size and liver lipids is shown in Table I. These data are similar to results obtained previously and corroborate these earlier observations(1). Body weight gain was depressed and liver size increased as a consequence of lithocholic acid ingestion. Lithocholic acid-fed chicks also had greatly increased plasma lipid levels.

The *in vitro* incorporation of acetate-1-^{14}C and glucose-U-^{14}C into cholesterol and fatty acids by liver slices was studied and these data are shown in Table II. Fatty acid synthesis from acetate and glucose by liver slices from lithocholic acid-fed chicks was significantly greater than for control chicks. Cholesterol synthesis from both the substrates used was increased by approximately 40% as a consequence of lithocholic acid ingestion; however, these differences did not attain statistical significance ($P>0.05$).

TABLE III. Oxidation of Glucose-1-^{14}C and Glucose-6-^{14}C by Liver Slices from Chicks Fed a Lithocholic Acid Free or Supplemented Diet.*

Addition to buffer	Dietary treatment: Control	Lithocholic acid supplemented	Probability of significance
	($m\mu$moles oxidized/100 mg tissue/3 hr)		
Glucose-U-^{14}C	410 ± 73†	680 ± 122	<.001
Glucose-1-^{14}C	253 ± 45	347 ± 27	<.001
Glucose-6-^{14}C	283 ± 72	333 ± 63	ns
C_1/C_6	.92 ± .14	1.06 ± .14	ns

* Incubation conditions as noted in Table II.
† Mean for 9 chicks ± S.D.

TABLE IV. Activity of Glucose-6-Phosphate Dehydrogenase, 6-Phosphogluconate Dehydrogenase and NADP Malic Dehydrogenase in Liver of Chicks Fed a Lithocholic Acid Free or Supplemented Diet.

Enzyme	Dietary treatment: Control	Lithocholic acid supplemented	Probability of significance
	μmoles of substrate converted/mg N/min at 30°		
Glucose-6-phosphate dehydrogenase	.053 ± .018*	.176 ± .072	<.001
6-Phosphogluconate dehydrogenase	.058 ± .024	.075 ± .037	ns
NADP malic dehydrogenase	.468 ± .135	.497 ± .186	ns

* Mean for 9 chicks ± S.D.

Liver slices from lithocholic acid-fed chicks oxidized more glucose-U-^{14}C and glucose-1-^{14}C than did tissue from control animals (Table III). However, the oxidation of glucose-6-^{14}C and the C-1/C-6 ratio of tissues from lithocholic acid-fed chicks were not significantly different from that of tissue from control chicks. The greater oxidation of glucose-1-^{14}C by tissue from lithocholic acid-fed chicks suggested an enhanced hexose monophosphate shunt activity (HMP); therefore, the activity of 2 HMP enzymes and malic enzyme in liver was determined. The data presented in Table IV demonstrate the higher G-6-PD activity of liver from the lithocholic acid-fed chicks. The activity of 6-PGD and malic enzyme was not altered.

The results of an experiment designed to study the turnover rate of cholesterol are shown in Fig. 1. There was a significantly lower cholesterol specific activity in the lithocholic acid-fed chicks as well as a decreased turnover time. The "half-life" of circulating cholesterol was determined from the regression equation for the specific activity-time relationship shown in Fig. 1. Such a computation gave a "half-life" for cholesterol of 57.9 hours for the control chicks as compared to 77.7 hours for the lithocholic acid-fed animals, an increase of 34% as a consequence of lithocholic acid ingestion.

If the administered cholesterol is assumed to be indistinguishable from the endogenous sterol, then the specific activity at zero time

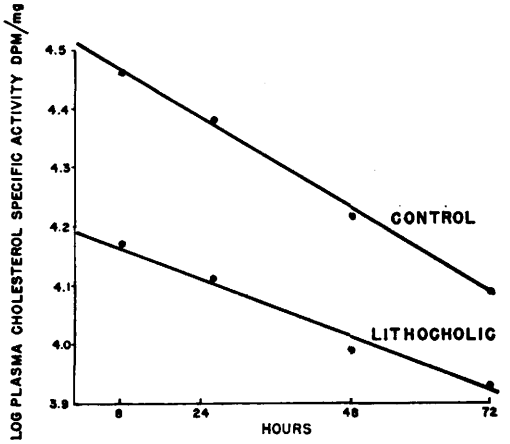

FIG. 1. Cholesterol turnover in control and lithocholic acid-fed chicks. Each point is the mean for 5 chicks. The calculated regression equations for the control and lithocholic acid curves are: Control, log specific activity = 4.5114—0.0059 hr; lithocholic acid, log specific activity = 4.1937—0.0038 hr.

(obtained by extrapolation) can be used to calculate the body pool size. Based on this assumption, the body pool was calculated to be 520 mg for the control chicks and 1040 mg for the lithocholic acid-fed chicks (an increase of 100%).

Discussion. The data presented suggest that the hypercholesterolemia resulting from the ingestion of lithocholic acid is a consequence of a less rapid turnover of cholesterol and perhaps an enhanced hepatic cholesterol synthesis. The enhanced fatty acid synthesis by liver tissue undoubtedly contributes to the observed lipemia. The exact mechanism by which these parameters are altered is obscure. However, these results might be explained by an impairment in the permeability of the hepatic cell to circulating lipids. The rate of cholesterol and fatty acid synthesis, as well as the conversion of cholesterol to bile acids by the liver, has been shown to be under feed-back control(10,11,12). Thus, the synthesis of cholesterol and fatty acids would be limited by the tissue level of these end products. Dietary lithocholic acid may impair the entry of lipid into hepatic cells, thereby stimulating fatty acid and cholesterol synthesis which would contribute to elevated plasma lipid levels. This speculated effect of lithocholic acid would account for a decreased conversion of cholesterol to bile acids and, thus, the longer "half-life" of cholesterol found in the lithocholic acid-fed chick. A decreased transport of cholesterol into the intracellular compartment of liver would result in a reduced supply of precursor for conversion to bile acids. The hypothesis suggested is similar to that recently proposed to explain the effect of stilbestrol on cholesterol metabolism in the rooster(13). The suggestion that dietary lithocholic acid somehow impairs the entry of circulating lipids into the liver cells enables a plausible explanation for the observed metabolic alterations in chicks ingesting this bile acid.

The increased glucose oxidation by liver slices from lithocholic acid-fed chicks is due to an increased oxidation of glucose-1-^{14}C without any change in the production of $^{14}CO_2$ from glucose-6-^{14}C, suggesting enhanced HMP shunt activity. This conclusion is further supported by the enhanced glucose-6-phosphate dehydrogenase activity in the liver of lithocholic acid-fed chicks. The enhanced HMP shunt activity would supply reduced coenzymes (NADPH) to support increased fatty acid and cholesterol synthesis (14) and would also supply ribose-5-phosphate for the increased ribonucleic acid synthesis necessitated by the hepatic cellular proliferation induced by the ingestion of lithocholic acid.

Several intriguing questions are raised by this and previous studies(1,2) on the effects of dietary lithocholic acid. The exact mechanism which triggers the ductular cell proliferation as well as the origin of the ductular cells remains obscure(14). Several parallels exist between the response to dietary lithocholic acid and to other types of intoxications such as ethionine and carbon tetrachloride (16,17). The possibility remains that the consequences of lithocholic acid ingestion in the chick reflect a response to a generalized stress condition. Studies are currently being pursued which will attempt to further elucidate the mechanism involved in the chick's response to lithocholic acid and to define the specific pharmacological effects of this bile acid.

Summary. Liver slices from chicks ingesting lithocholic acid incorporated more acetate-1-^{14}C and glucose-U-^{14}C into cholesterol and fatty acids than did similar preparations from control chicks. The oxidation of glucose-U-^{14}C and glucose-1-^{14}C by liver slices of lithocholic acid-fed animals was enhanced. The activity of glucose-6-phosphate dehydrogenase was greater in the liver of lithocholic acid-fed as compared to control chicks, indicating an increased pentose shunt pathway activity. Cholesterol turnover studies demonstrated an increased "half-life" for chicks fed lithocholic acid, 77.7 *versus* 57.9 hours for lithocholic acid-fed and control chicks, respectively.

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1. Leveille, G. A., Sauberlich, H. E., Hunt, R. D., Proc. Soc. Exp. Biol. and Med., 1963, v114, 334.
2. Hunt, R. D., Leveille, G. A., Sauberlich, H. E., ibid., 1963, v113, 139.
3. Leveille, G. A., Hanson, R. W., J. Lipid Res., in press.
4. Horecker, B. L., Smyrniotis, P. Z., Methods in Enzymol., 1955, v1, 323.
5. Ochoa, S., ibid., 1955, v1, 699.
6. Johnson, M. J., J. Biol. Chem., 1941, v137, 575.
7. Meier, J. R., Siperstein, M. D., Chaikoff, I. L., ibid., 1952, v198, 105.
8. Kabara, J. J., J. Lab. Clin. Med., 1957, v50, 146.
9. Leveille, G. A., Sauberlich, H. E., Proc. Soc. Exp. Biol. and Med., 1963, v112, 300.
10. Beher, W. T., Baker, G. D., Anthony, W. L., Beher, M. E., Henry Ford Hosp. Med. Bull., 1961, v9, 201.
11. Siperstein, M. D., Am. J. Clin. Nutrition, 1960, v8, 645.
12. Lynen, F., Matsuhashi, M., Numa, S., Schweizer, E., Chapter in The Control of Lipid Metabolism, Popjack, G., Grant, J. K., Eds., Academic Press, N. Y., 1963.
13. Sakakida, H., Shediac, C. C., Siperstein, M. D., J. Clin. Invest., 1963, v42, 1521.
14. Tepperman, J., Tepperman, H. M., Pharmacol. Rev., 1960, v12, 301.
15. Rubin, E., Exp. Mol. Pathol., 1964, v3, 279.
16. Popper, H., Kent. G., Stein, R., J. Mt. Sinai Hosp., 1957, v24, 551.
17. Isselbacher, K. J., Jones, W. A., Gastroenterol., 1964, v46, 424.

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Uptake of Reduced Folic Acid by Chicken Liver Enzyme. (30675)

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The information whether folic acid in serum occurs free or in a combination with proteins is both meager and controversial. Condit and Grob(1) by using various methods (*e.g.*, electrophoresis and bioautography, paper chromatography, equilibrium dialysis), concluded that folic acid in plasma is not bound to protein, while Johns *et al*(2) using H^3PGA (H^3-pteroylglutamate) found that as much as 64% of PGA in plasma is protein-bound. A highly dissociable protein-bound PGA conjugate was found to occur in yeast(3).

In our laboratory, while investigating the binding by serum of vit. B_{12}(4), which is functionally related to folic acid, a liver extract preparation was found to restore the binding by serum which was inactivated by boiling(5). Experiments were thus designed to investigate whether a similar mechanism operates in relation to PGA. As an outcome of these studies we have found that liver extract, and not serum, is active in binding H^3PGA. This finding led us to investigate further the nature of the uptake. Some of our results are presented here.

Materials and methods. Tritiated pteroylglutamic acid (H^3PGA, specific activity 1 μg/0.263 μC, purchased from the Radiochemical Centre, Amersham, England) was used. Reduced nicotinamide adenine dinucleotide phosphate (NADPH) and its oxidized form (NADP), adenosine triphosphate (ATP) were obtained from the Nutritional Biochemical Corp., Cleveland, Aminopterin from Mann Research Laboratories, New York, Norit A from Fisher Scientific Co., and 2-mercapto-ethanol was obtained from Matheson, Coleman and Bell Co.

The chicken liver cell-free extract was prepared according to Chanarin and Bennett(6). The uptake of H^3PGA was determined in the Packard Tricarb Liquid Scintillation Spectrometer model 314E. A mixture of 2 scintillation solutions was used: a) a dioxane scintillation solution and b) a toluene scintillation solution. Their composition was as follows: a) 2,5 diphenyl-oxazole—7 g, naphthalene—50 g and 1-4-di-[2-(5-phenyloxa-

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