without the chronic loss from the body of the vital substances carried by the lymph. Perhaps certain forms of permanent organ damage, including fibrosis, might thus be prevented.

Summary. Chronic impairment of hepatic lymph drainage in the dog resulting from resection of a segment of thoracic duct and/or constriction of the superior vena cava leads to discernible changes in liver histology. These changes, though not quantitatively marked, are comparable to those seen in early cardiac fibrosis of the liver (so-called "cardiac cirrhosis"), and support the hypothesis that some of the changes in the liver associated with venous congestion are due to impairment of lymph drainage.

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Effect of Nicotinic Acid on Pool Size and Turnover of Taurocholic Acid in Normal and Hypothyroid Dogs.* (31329)

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The mechanism of the hypocholesterolemic action of large doses of nicotinic acid has not been established. Since the conversion of cholesterol to bile acids is an important step in cholesterol catabolism, we investigated some parameters of bile acid metabolism in normal and hypothyroid dogs before and during the administration of nicotinic acid. Biologic half-life, pool size, and turnover of taurocholic acid (TC), the only primary bile acid present in significant amounts in dog bile (1,2), were determined by labeling the cholic acid pool with cholic-^{14}C acid and following the rate of disappearance of the radioactive label from the bile.

Methods. Female mongrel dogs, weighing 11 to 15 kg, were used. They were fed a commercial meat-type diet to maintain a steady weight. Total plasma cholesterol concentrations were measured by a modification of the Zak method(3).

Multiple small samples of bile were obtained in two different ways. In 2 animals the common bile duct was cannulated proximally and distally with polyethylene tubes which were brought out subcutaneously to the back of the animals and connected *via* a short loop to keep the enterohepatic circulation intact. Bile samples were obtained by interrupting the circuit. (Long-term maintenance of these bile fistulas proved to be difficult.) In 3 dogs a Thomas cannula(4) was placed into the duodenum opposite to the papilla of Vater, and bile was sampled from the duodenum after stimulation of bile flow with cholecystokinin (generously supplied by E. R. Squibb & Sons, New York).

The bile acid pool was labeled by adminis-

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tration of 10 to 30 μc of cholic-carboxyl-^{14}C acid (specific activity, 2.53 mc/mmole) (Tracerlab; Waltham, Mass.) in approximately 50 ml of 10% ethanol by stomach tube. Bile was sampled daily, from fasting dogs, over a period of 8 to 12 days after administration of the tracer dose. For analysis, 0.5 to 1 ml of bile was used. Quantitative thin-layer chromatography (butanol-acetic acid-water, 10:1:1) for isolation and determination of TC was carried out according to recently described techniques(5). Radioactivity was measured by liquid scintillation spectrophotometry by a modification of the method described by Snyder and Stephens (6). The specific activity of the TC was calculated as counts per minute per milligram. The biologic half-life of TC was obtained from a semilogarithmic plot of specific activity against time, drawn to the best visual fit. Pool size and turnover were then calculated according to Lindstedt(7).

Nicotinic acid was administered orally in single daily doses of approximately 100 mg/kg for 2 weeks prior to and during the periods of bile sampling. An attempt was made to induce hypercholesterolemia in 2 dogs by intravenous administration of 10 mc of ^{131}I. When no significant increase in the plasma cholesterol concentration was apparent after 10 weeks, 250 mg/day of propylthiouracil was given orally in divided doses.

Results. Fig. 1 shows the disappearance rates of labeled TC from the bile of 2 animals. The semilogarithmic plot of the specific activity of TC *vs* time in days demonstrates linearity regardless of the method of sampling used. The faster disappearance of radioactivity from the pool after cholecystectomy indicates a shortened biologic half-life.

Table I summarizes the data obtained in 5 dogs. In 4 dogs with intact gallbladder the biologic half-life of TC ranged from 1.3 to 2.3 days, the pool size from 1.07 to 1.21 g, and the turnover from 0.33 to 0.67 g/day. After cholecystectomy in dog E, the half-life was shorter, the pool size was decreased, but the turnover was increased only slightly. In the other cholecystectomized dog, D, there was a similarly short half-life with a decreased pool and a turnover in the normal range.

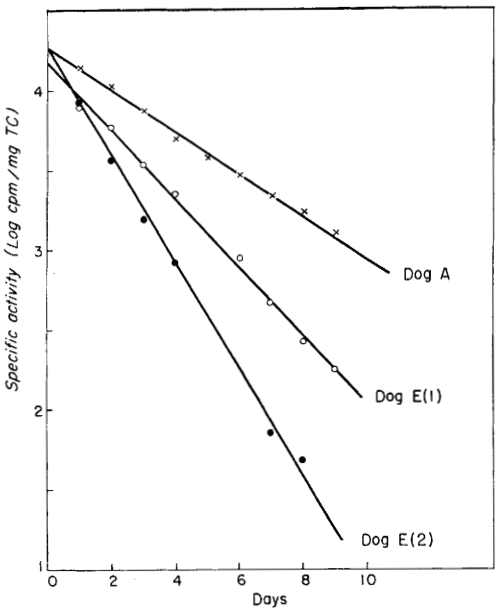

FIG. 1. Semilogarithmic plot of specific activity of taurocholic acid (TC) *vs* time in days following oral administration of cholic-^{14}C acid. Dog A had polyethylene tubes in proximal and distal parts of common bile duct. Dog E had Thomas cannula and was studied twice, before (*1*) and after (*2*) cholecystectomy.

The effect of nicotinic acid on these parameters was investigated in 2 dogs, intact dog, C, and a cholecystectomized dog, D. Each dog was studied twice, first in the normal, euthyroid state and then in the hypercholesterolemic, hypothyroid state.

Fig. 2 depicts the plasma cholesterol levels of these 2 dogs during the entire 30-week study period. The values decreased only slightly after 4 weeks of treatment with nicotinic acid; they increased significantly about 14 weeks after administration of ^{131}I, which

TABLE I. Effect of Cholecystectomy on Pool Size and Turnover of Taurocholic Acid in Dogs.

Dog	Half-life (days)	Pool (g)	Turnover (g/day)	Plasma cholesterol (mg/100 ml)
Normal				
A	1.3	1.21	.67	149
B	2.3	1.08	.33	230
C	2.1	1.07	.36	174
E (1)	1.4	1.08	.54	171
Cholecystectomy				
E (2)	.85	.91	.73	146
D	.75	.33	.31	89

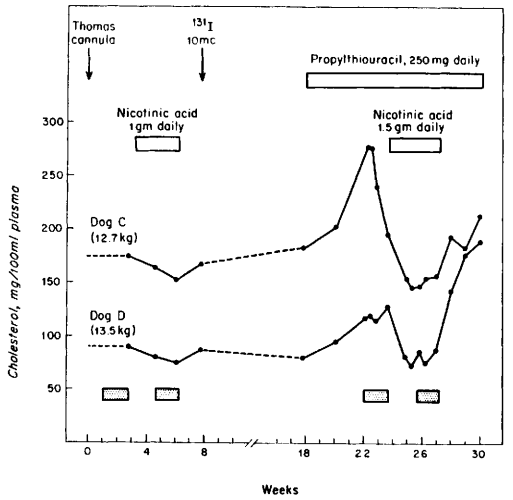

FIG. 2. Effect of ^{131}I, propylthiouracil, and nicotinic acid on plasma cholesterol levels of 2 dogs. Stippled bars indicate periods when pool size and turnover of taurocholic acid were determined.

was 4 weeks after the start of treatment with propylthiouracil. Cholesterol values returned to base-line levels 2 weeks after the start of daily administration of 1.5 g of nicotinic acid; they began to increase again after the nicotinic acid regimen was discontinued.

Pool size and turnover of TC were determined 4 times during this period: before and during treatment with nicotinic acid in the normal state, and before and during treatment in the hypercholesterolemic state.

The decrease of the specific activity of TC in the bile of these 2 dogs during the different study periods is shown in Fig. 3. The slopes of the curves are less in the hypothyroid state when compared with the normal state in both dogs, and there is a lower specific activity at time zero in both instances. The differences between the slopes for the periods before and during treatment with nicotinic acid in the normal state are of questionable significance. For the hypercholesterolemic state, no difference could be seen in the curves before and during treatment with nicotinic acid. It is noteworthy that the decay of radioactivity in the cholecystectomized dog, D, was much faster than in dog C, again indicating a short biologic half-life of TC.

The data for half-life, pool size, and turnover together with the mean cholesterol levels during each study period are summarized in Table II. Although nicotinic acid has a striking effect on the hypercholesterolemia induced by hypothyroidism(2), no alteration of the dynamics of bile acid metabolism was

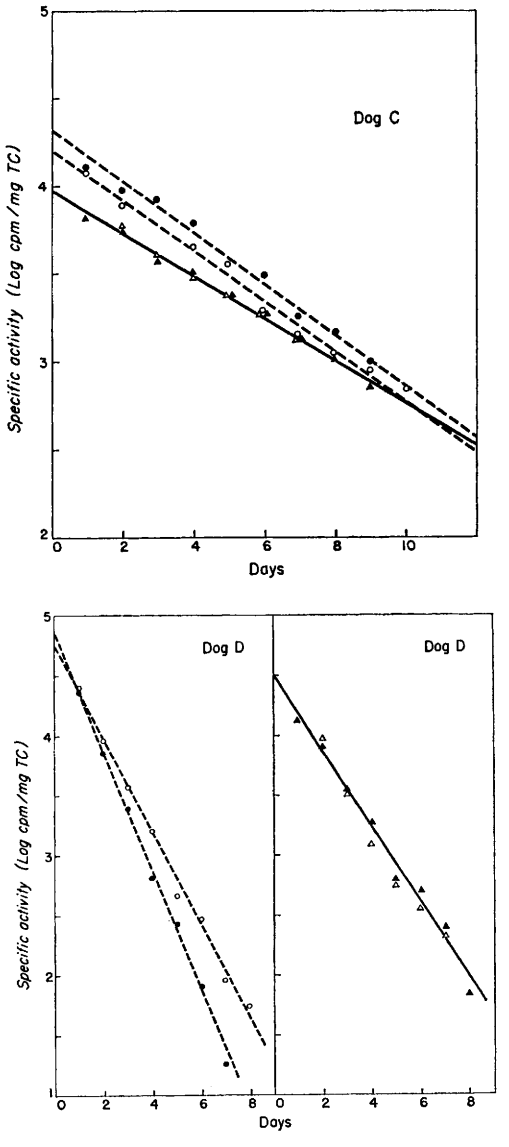

FIG. 3. Semilogarithmic plot of specific activity of taurocholic acid (TC) *vs* time in days following administration of cholic-^{14}C acid to dogs. Dog C, normal; dog D, cholecystectomized. Broken lines = euthyroid state; solid lines = hypothyroid state; open symbols = before treatment with nicotinic acid; solid symbols = during treatment with nicotinic acid.

TABLE II. Effect of Nicotinic Acid on Plasma Cholesterol Level and Taurocholic Acid Metabolism of 2 Dogs in Euthyroid and Hypothyroid States.

State	Half-life (days)	Pool (g)	Turnover (g/day)	Plasma cholesterol (mg/100 ml)
		Dog C		
Euthyroid	2.1	1.07	.357	174
" + nicotinic acid	2.1	.97	.320	160
Hypothyroid	2.4	2.02	.585	260
" + nicotinic acid	2.5	2.04	.565	150
		Dog D (cholecystectomized)		
Euthyroid	.75	.33	.306	89
" + nicotinic acid	.65	.28	.295	77
Hypothyroid	1.0	.65	.450	120
" + nicotinic acid	1.0	.69	.480	80

demonstrated during treatment with nicotinic acid, either in the euthyroid state or in the hypothyroid state. Although pool size and turnover of TC in the euthyroid state are slightly lower during treatment with nicotinic acid, the differences do not appear to be significant.

Compared to the euthyroid state, the hypothyroid state was characterized by a prolonged half-life, an almost doubled pool size, and an increased daily production of TC. This pattern remained unaltered even though the plasma cholesterol concentrations returned to normal during administration of nicotinic acid.

Discussion. There is disagreement in the literature regarding the effect of nicotinic acid on plasma cholesterol levels in dogs fed various diets(2,8-13). Most investigators have concluded that nicotinic acid has little or no effect on the plasma cholesterol concentrations in normal dogs fed a regular diet. Kottke and co-workers(2) reported a marked hypocholesterolemic effect when nicotinic acid was given to hypothyroid dogs which had increased plasma cholesterol levels. Their findings are confirmed by the present study. Zanetti and Tennent(13) observed that nicotinic acid counteracted the hypercholesterolemia associated with pseudopregnancy in female dogs.

Studies of the dynamics of bile acid metabolism, by labeling the bile acid pool, have been done in rats(14,15), rabbits(16,17), and humans(7,17-19) under various conditions. In a recent study, Playoust and associates (20) found that the half-time for loss of radioactivity from bile in normal dogs was about 1 day when taurocholate-^{14}C was given intravenously. Although these authors did not measure the specific activity of TC itself, which is necessary for quantitative calculations, this value compares favorably with our results.

Our preliminary findings that the dynamics of bile acid metabolism are altered after cholecystectomy are of interest. Removal of the gallbladder results in a shortened biologic half-life of TC. In the one instance in which studies were done before and after cholecystectomy, the size of the total miscible pool decreased slightly and the turnover of TC increased.

The size of the total miscible pool of TC in 4 normal dogs with intact gallbladder ranged from 1.07 to 1.21 g. In dog E and in 2 other dogs, the total amounts of TC present in the gallbladder at the time of cholecystectomy, when the dogs had fasted for 24 hours, were 0.9, 1.1, and 1.3 g. This indicates that, in the fasting state, probably 90% or more of the bile salts are present in the gallbladder. Three weeks after cholecystectomy in dog E, the pool size decreased only from 1.08 to 0.91 g. It seems reasonable to assume that a new distribution equilibrium for the bile salts develops with little change in the total bile acid pool. Distribution studies with labeled bile acids in the rat(15) have revealed that about 70 to 90% of the total bile acid pool is found in the small intestine of this animal which lacks a gallbladder. Studies in rabbits(16) have demonstrated that the percentage of labeled bile acid present in liver and gallbladder may range anywhere from 2.7 to 65.8%.

Abell and associates(21) reported that administration of thiouracil to dogs fed a stock diet low in cholesterol did not produce detectable changes in the excretion of cholesterol and bile acids. Turnover studies of bile acids have been done in rats(22,23) and in humans(19) with abnormal thyroid function. Strand(23) reported that, when treated with propylthiouracil, rats with intact enterohepatic circulation showed about the same biologic half-life, pool size, and daily production of bile acids as did normal rats. Hellström and Lindstedt(19) studied a group of myxedematous patients before and after treatment with thyroxine and found an increased mean half-life of cholic acid and a decreased pool size and turnover in the hypothyroid state. We found a longer half-life and an increased pool size and turnover of TC in 2 hypothyroid dogs compared with euthyroid dogs. Because the hypothyroidism was induced by a combined treatment with ^{131}I and propylthiouracil and because of the species differences, it is difficult to compare our results with the previous reports.

The influence of nicotinic acid on biologic half-life and turnover of bile acids has not been reported previously. Kottke and associates(2) made the observation that nicotinic acid increased the size of the circulating pool of TC in 2 normal and one hypothyroid dog during the initial 5 to 7 days of nicotinic acid therapy. In their study the circulating pool was determined by a nonisotopic technique which made it impossible to keep the enterohepatic circulation completely intact. In contrast, the present studies were done in the third and fourth weeks of nicotinic acid therapy, when the plasma cholesterol level had reached its lowest value, and with only minimal disturbance of the enterohepatic circulation.

Neither in the euthyroid nor in the hypothyroid state did nicotinic acid have any significant effect on pool size, biologic half-life, or turnover of TC in the dog under the conditions of our study. This is in accord with findings of Miller and co-workers(24) in man. The authors concluded that nicotinic acid does not lower plasma cholesterol levels by increasing the fecal excretion of bile acids or steroids.

Summary. The rate of disappearance of taurocholic-^{14}C acid from bile was studied in a group of dogs after oral administration of cholic-carboxyl-^{14}C acid. The specific activity of taurocholic acid was determined in multiple small samples of bile by quantitative thin-layer chromatography and liquid scintillation counting; biologic half-life, pool size, and turnover of taurocholic acid were calculated. Hypothyroidism, induced in 2 dogs by administration of ^{131}I and propylthiouracil, increased the plasma cholesterol concentration and resulted in a prolonged half-life, an increased pool size, and an increased turnover of taurocholic acid. Nicotinic acid did not alter the dynamics of the metabolism of taurocholic acid in either the normal or the hypothyroid dogs, despite its marked hypocholesterolemic effect in the hypothyroid dog.

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Characteristics of Lytic and Non-Lytic Derived Strains of *Pseudomonas aeruginosa.** (31330)

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One of the common cultural characteristics associated with many strains of *Pseudomonas aeruginosa* is the spontaneous production of turbid plaque-like erosions on themselves when cultivated on tryptone agar media(1,6, 10). Lysis in most strains is accompanied by a metallic iridescence, while other strains form flecks of metallic patches on agar media (4), but do not exhibit visible lysis. The mechanism by which lysis occurs is presently unknown since little or no infectious phage, bacteriocines, or lytic enzymes appear to be detectable in individual plaques or in the harvested growth media(2). Consequently, the term "auto-plaque" has been adopted to describe this self-lytic phenomenon and to differentiate it from the plaques obtained with phage plated on sensitive indicator strains, the phage carrier state(9), and virulent mutant phage(5). Previous studies by Berk and Gronkowski(2) indicated that non-lytic strains (AP^-) could spontaneously revert or give rise to lytic strains (AP^+). However, detection of naturally occurring AP^- cells in lytic cultures has not been successful, nor have mutagenic and curing technics been effective in converting lytic cells to the non-lytic state(3). However, studies to be described herein indicate that under certain environmental conditions, AP^- cells can be isolated from AP^+ cultures. Therefore, the purpose of this report is to compare some of the cultural characteristics of 7 AP^- strains with their respective lytic parent cultures.

Materials and methods. The cultures of *P. aeruginosa* used in this study were obtained from various clinical sources and the departmental stock culture collection. Each culture was streaked for isolated colonies and a pure culture of each phenotype was obtained by picking individual colonies and re-streaking on tryptone agar media. Cultures which produced auto-plaques in areas of confluent growth and in individual colonies within 16 to 48 hours were labelled AP^+ to differentiate them from stable cultures which did not exhibit visible lysis (AP^-) at 37°C.

All strains examined for auto-plaque formation were cultured on a medium composed of 2% tryptone (Difco), 1% glucose, and 0.5% sodium chloride, while all stock cultures were maintained on a rehydrated tryptose medium (Difco) at 4°C after an initial incubation at 37°C for 24 hours. Tryptose agar was also used for determination of pigment production at 37°C and subsequently at 4°C. Chlororaphin and oxychlororaphin crystal formation was observed to occur on tryptone-glucose agar or broth media after 5 to 7 days incubation at 37°C, but was not detectable on tryptose medium(7).

Auto-plaque stimulation was demonstrated

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