increase in plasma LDH activity in the normal animal is that the LDH agent destroys, damages, or alters the permeability of cells. A second possibility is that the LDH agent increases enzyme production by altering the metabolism of cells. Thirdly, the LDH agent may increase the activity of already existing enzyme or activate a proenzyme. Lastly, the LDH agent may impair the rate of clearance of an enzyme from the plasma. Since the LDH agent acts in the non-tumor-bearing animal, there is no reason to assume that it acts through a different mechanism in the tumor-bearing animal. It is more likely that in the tumor-bearing animal infection with the LDH agent results in higher plasma enzyme activity due to a greater abundance, susceptibility, or enzyme content of the target cells. Experiments are being designed to test some of these possibilities.

Summary. Normal and tumor-bearing mice were experimentally infected with the LDH agent and followed for changes in plasma LDH and GOT activity. 1) Injection of the LDH agent into normal animals produced a 4- to 5-fold increase in plasma LDH activity within 72 hours and a small increase in plasma GOT. 2) Plasma from animals bearing the uninfected plasma-cell tumor SS (70429) showed a gradual increase in both LDH and GOT activity which paralleled the gross increase in tumor size. Transfer experiments failed to reveal a transmissible LDH agent. 3) The tumor-bearing animals which had been infected with the LDH agent showed up to 8 times more plasma LDH activity than the uninfected tumor-bearing animals and up to 88 times more activity than the normal animals. 4) Up to a 22-fold difference in plasma LDH activity was noted between the infected non-tumor-bearing animals and the infected tumor-bearing animals. Serial dilution of plasma from these 2 groups failed to reveal a difference in titer of the LDH agent.

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Experimental Cholelithiasis in the Guinea Pig.* (27401)

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In addition to many other biologic effects, the occurrence of flocculant precipitate and small firm masses in gall bladder bile followed parenteral injection of Klebsiella polysaccharide in the guinea pig(1). The initial gall bladder distension and apparent stasis, the edema of the gall bladder and adjacent structures resembled acute cholecystitis in man. Following intravenous injection of C^{14}-labeled polysaccharide into guinea pigs, considerable label was found within protein-rich ascitic and retroperitoneal fluid, and label appeared in the bile(2). It was not known whether the label in bile represented excreted metabolites of the injected polysaccharide or the exudation of polysaccharide-containing

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plasma into the gall bladder. In either event, the labeled material might participate directly in physicochemical changes in the bile resulting in formation of precipitate. The present study concerns the nature of the labeled moieties in bile, their enterohepatic cycling and the composition of the bile precipitate and small stones.

Materials and methods. The capsular polysaccharide, biosynthetically labeled with C^{14} and obtained from *Klebsiella pneumoniae* as described previously(2) was prepared as a 1% solution in 0.15 M NaCl and injected by ear vein, 0.50 mg/100 g body weight, into young guinea pigs of either sex and 300-350 g each in weight. Animals were supplied with Purina rabbit chow, leafy green vegetables and water *ad libitum*. Animals were killed 1 and 2 days after the single injection of polysaccharide, the common bile duct immediately clamped and bile aspirated from the gall bladder.

The individual precipitates were collected, washed with water, dried *in vacuo* over $CaCl_2$ at 59°C, weighed and dissolved in hot chloroform:ethanol:acetone (1:1:1). Aliquots of such solutions were used for determination of a) bile acids by chromatography(3) and spot tests with phosphomolybdic and phosphotungstic acids(3), b) cholesterol by paper chromatography, spot tests as above and elution of corresponding zones of non-sprayed strips followed by quantitative chemical determination(4), c) bile pigment by redissolving dried aliquot in 5% $NaHCO_3$ and measuring with Beckman DU spectrophotometer (5), and d) C^{14} by application to aluminum planchets and counting in windowless flow counter. The fluid bile samples separated from the precipitate were pooled according to the 2 time intervals.

The bile and bile precipitate was also examined microscopically and microsections prepared of the gall bladders.

Enterohepatic cycling of labeled moieties was studied by catheterization of both the gall bladder and the common bile duct distal to the site of its ligation, as previously described(3,6). Bile was collected at hourly intervals from the gall bladder catheters of animals given C^{14} labeled Klebsiella polysaccharide by the intravenous route. This bile was introduced into the duodenum of the animals by means of their common bile duct catheters and bile from their gall bladder catheters was collected over a period of 6 to 12 hours. In all animals the amount of bile collected each hour was replaced by an equal volume of bile introduced intraduodenally through the common duct catheter, normal bile making up any deficit in injected compared to collected bile. C^{14} in all bile samples was determined as indicated above.

Bile collected over 12-hour period from gall bladder catheters in guinea pigs given C^{14} labeled Klebsiella polysaccharide by intravenous and by intraduodenal routes was placed in cellophane tubing and dialyzed against water for 2 days. The original labeled polysaccharide was subjected to similar dialysis. Precipitin tests with labeled materials and rabbit anti-Klebsiella antiserum were carried out as described previously(7).

Results. One and 2 days after intravenous injection of labeled polysaccharide, the gall bladders were usually distended and their walls edematous. In about one-half of the animals the clear bile contained flocculant green or pale yellow-green precipitate. Occasionally such material was pendulously attached by thin clear strands to the mucosa. Microscopically, a few intact epithelial cells were seen within the amorphous and crystalline material. Microsections of the gall bladders from animals given the single injection of polysaccharide and killed at 10 days, disclosed proteinaceous edema fluid and scattered polymorphonuclear leukocytes within the mucosa and muscularis (Fig. 1). Some of the uniform fine cytoplasmic particles, which characterize the mucosal epithelium, stained with Alcian blue while other intermingled particles stained with periodic acid-leucofuchsin. Rarely, the gall bladders of some animals showed groups of lymphocytes, suggesting a chronic inflammatory process antedating the injection of the polysaccharide.

In contrast to the considerable C^{14} content of tissues after intravenous injection of labeled polysaccharide, little radioactivity was present in tissues after the intraduodenal introduction of this product (Table I). Dur-

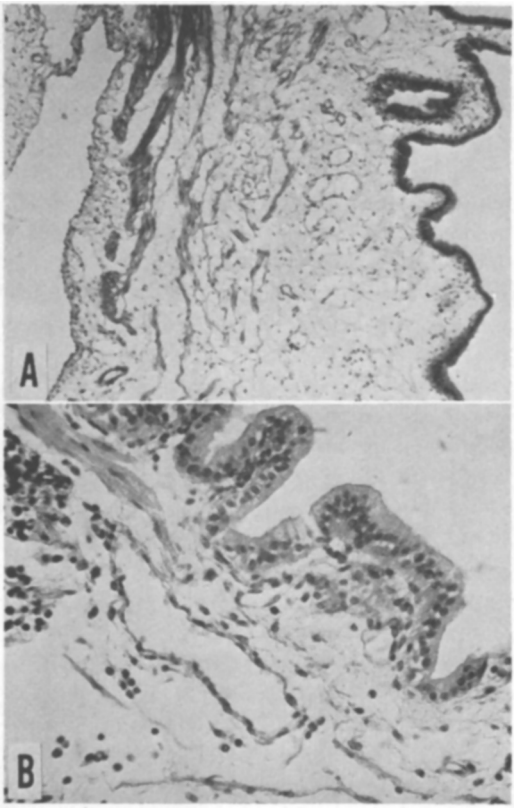

FIG. 1. A. Gall bladder wall one day after single intrav. inj. of Klebsiella polysaccharide, showing edema, vascular dilatation, sparse polymorphonuclear leucocytic infiltration and intact mucosal epithelium. H & E × 42. B. Similar changes as above with focal aggregates of polymorphonuclear leukocytes. H & E × 176.

ing the first 12 hours only .38% of the C^{14} was excreted in the urine following the intraduodenal injection of labeled polysaccharide as compared to the 2.58% following the intravenous injection. Only traces of C^{14} were present in the feces after injection of polysaccharide by either route, but similar amounts of label appeared in the bile (Fig. 2). After intraduodenal injection of labeled polysaccharide, all of the label appearing in bile was dialyzable; after intravenous injection, the 7% of biliary label which was non-dialyzable did not precipitate with anti-Klebsiella sera. None of the original labeled polysaccharide was dialyzable under similar conditions.

The C^{14} excreted in bile after intravenous injection did not undergo enterohepatic cycling. Label failed to appear in the gall bladder catheters of animals given label-containing bile intraduodenally (Fig. 2). Only traces of C^{14} were found in the tissues of these guinea pigs, no label was detected in the feces, and .77% to 1.28% of the C^{14} was excreted in the urine within the 12-hour period of observation.

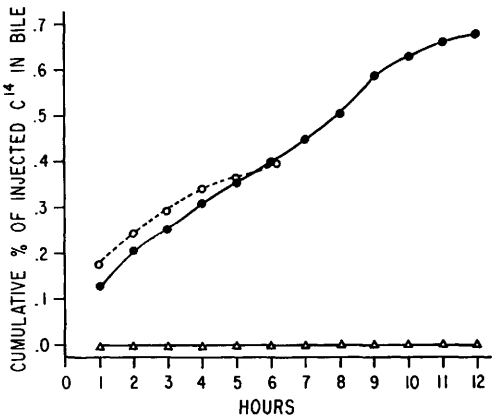

FIG. 2. C^{14} (cumulative %) appearing in gall bladder bile after intrav. inj. of labeled Klebsiella polysaccharide (●—●), after intraduodenal inj. of labeled polysaccharide (○—○) (6 hr period only). No C^{14} was excreted in bile after intraduodenal inj. of labeled bile obtained from donor animals given C^{14}-labeled polysaccharide intrav. (△—△).

No C^{14} was found in the precipitate recovered from gall bladder bile of animals killed 1 and 2 days after intravenous injection of bacterial polysaccharide. The precipitate contained no bile acids but consisted predominantly of cholesterol with some bile pig-

TABLE I. Comparison of Tissue Distribution of C^{14} One Day after Intravenous and Intraduodenal Introduction of Labeled Klebsiella Polysaccharide in Guinea Pigs with Biliary Tract Catheters.

	No. animals	Plasma, %/ml	Liver, %/g*	Spleen, %/g	Adrenal, %/g	Mesenteric lymph node, %/g
Intravenous	10	.62 ±.28	2.02 ±.52	6.90 ±2.00	6.42 ±1.16	.43 ±.25
Intraduodenal	5	.0009 ±.0005	.15 ±.06	.04 ± .02	.09 ± .04	.27 ±.15

* Dry wt.

TABLE II. Composition of Precipitate in Gall Bladders of Guinea Pigs 1 and 2 Days after a Single Intravenous Injection of Klebsiella Polysaccharide.

	Day 1	Day 2
	%	
Cholesterol	85.0 ± 3.5	84.1 ± 6.7
Pigment	11.0 ± 2.7	14.6 ± 2.0
C^{14}	0	0
Bile acids	0	0

ment (Table II). In fluid bile some of the label was present in the conjugated bile acids but no label was found in the free bile acids obtained by the hydrolysis of these conjugates.

Comments. The precipitate and stones formed in the bile of guinea pigs following parenteral injection of Klebsiella polysaccharide was similar in composition to many biliary calculi in man(8). The initial edema and mild leukocytic infiltration of the gall bladder wall following injection of polysaccharide were not unlike morphologic changes of acute cholecystitis. We have observed similar precipitate after various suppurative bacterial lesions. Gall stones consisting of calcium phosphate and some cholesterol were described by Okey(9) in guinea pigs fed diets rich in riboflavin, cholesterol and fat.

Since little, if any, labeled polysaccharide was present in bile, the polysaccharide *per se* could not have directly produced the physicochemical alterations leading to the cholesterol and pigment precipitate. The lack of continued enterohepatic cycling of the biliary C^{14} derived from the labeled polysaccharide would exclude a cumulative effect of such derivatives upon the bile or bile ducts. The labeled moieties in bile were not present in the precipitate. The bile acids produced after injection of the bacterial polysaccharide, however, may be altered. Unpublished studies suggest that the ratio of the 3 bile acids was modified, and that the small fraction of C^{14} in the conjugated bile acid was neither in glycine nor taurine. Such changes in bile acids might significantly alter the physicochemical relationship of bile constituents. It is also possible that primary injury of gall bladder walls by the intravenously injected polysaccharide was followed by selective absorption of bile salts which then led to the precipitation of cholesterol and pigment.

Tissue uptake of C^{14} from labeled bacterial polysaccharide introduced into the gut was far less than the tissue uptake of polysaccharide introduced by intravenous, intraperitoneal or subcutaneous routes(10). After intravenous injection a considerable portion of the Klebsiella polysaccharide in tissues has been shown to retain its original solubility and haptenic characteristics(2). Apparently, after intraduodenal injection, Klebsiella polysaccharide was not absorbed as such but degraded within the gut and absorbed as small moieties, some of the label having been incorporated in mesenteric nodes and liver, some excreted in urine and a comparable amount having made a single enterohepatic cycle.

The present studies have not determined whether the labeled biliary material represents a metabolic derivative of the 3 biosynthetically labeled monosaccharide constituents—glucose, glucuronic acid and rhamnose (Jones, Radcliffe and Linker—unpublished studies), or another component or admixed moiety present in trace quantity in the injected polysaccharide.

Summary. Within 1 day after intravenous injection of C^{14}-labeled Klebsiella polysaccharide, cholesterol and pigment precipitate and small firm masses appeared in the gall bladder bile of guinea pigs. No C^{14} was present in the precipitate and less than 1% of the label from the injected bacterial polysaccharide was excreted in the bile during the first 12 hours. Since bacterial polysaccharide could not be identified in the bile, the precipitate was not due to the direct physicochemical effect of this material. The label in bile underwent a single enterohepatic cycle and some of this label was conjugated with bile acids.

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