Solubility of Human Gallstones in Primate Gallbladder.* (25731)

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Since human gallstones are quite different from those found in animals in regard to high cholesterol content, and since they are dissolved when placed in the gallbladder of such animals as the dog(1), pig, sheep and goat (2), the work on experimental animals cannot be freely translated to man. The only significant difference between bile of man and of experimental animals is found in degree of saturation with cholesterol, *i.e.*, human bile is highly saturated and others are not(3). Since it is not feasible to use human subjects in some of our studies, our effort has been concentrated on finding an animal species in which bile is completely or almost completely saturated with cholesterol as in man(4). Primates aroused our interest because of other similarities to man, and because of their use in studying cholesterol metabolism in relation to atherosclerosis. The present communication demonstrates that human gallstones are less soluble in monkey gallbladder than in other experimental animals, suggesting its bile is more saturated with cholesterol than that of other animals. Comparison of bile constituents of man, monkey and dog is presented together with rate of solution of gallstones placed in the gallbladder.

Methods and materials. Weight of monkeys used in this investigation is about 3.5 lb for Cebus monkey (*Cebus capuchinus*) and 7 to 8 lb for Rhesus monkey (*Macaca mulatta*). They were fed standard monkey chow diet. Under pentobarbital anesthesia the gallbladder was opened by small incision in the fundus. A human gallstone, weighing 100 to 600 mg was inserted into the gallbladder and the incision closed with fine cotton suture in double layers. Care was taken not to pass the suture through the mucosa so as not to cause leakage of bile. The stones used were multiple cholesterol stones containing over 98% of cholesterol obtained from the same gallbladder. After varying lengths of time gallbladders were reopened, the stones recovered and weighed after brief drying in air. Normal human hepatic bile was obtained from choledochostomy tube of a case operated upon after abdominal trauma and pooled. Human gallbladder bile samples were obtained from several patients suffering from diseases other than that of the hepatobiliary system during laparotomy. Several bile constituents, such as bile salts, cholesterol and phospholipids were analyzed, using methods described previously(4).

Results. Dissolution of human gallstones in monkey gallbladder. Eight Rhesus monkeys and 6 Cebus monkeys were used. The results are summarized in Table I, together with dissolution rate of stones in other experimental animals. Human gallstones are much less soluble when placed in monkey gallbladder than in those of other experimental animals, such as dog, hog, sheep and goat. In one out of 8 Rhesus monkeys and 3 out of 6 Cebus monkeys it was found, during second operation, that the cystic duct was apparently obstructed by the stone previously inserted in gallbladder and no bile was found in the gallbladder. Consequently, no decrease of weight of stone was expected in these cases.

Comparison of major constituents of bile from man, monkey and dog. The results of analysis of monkey bile in comparison with those from man and dog are summarized in Table II. There is no fundamental difference in composition between human, monkey and dog bile, except that human and monkey bile have much higher cholesterol concentration.

Identification of bile salts in monkey bile by paper chromatography. Bile salts were isolated from bile using countercurrent distribution according to method of Wiggins and Wootton(6). After alkaline hydrolysis of isolated bile salts, they were subjected to paper partition chromatography according to

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TABLE I. Solution of Human Gallstones Placed in Gallbladders of Primate and of Usual Experimental Animals.

Animal		Days elapsed	Original wt of inserted gallstone, mg	Wt loss	
				In mg	In %
		Experimental animals			
Dog	1	30	1,204.4	364.4	33.0
	2	100	1,217.9	756.6	62.2
*Hog	1	37	155	37	23
	2	94	159	94	59
*Sheep	1	114	834	527	63
	2	142	888	852	96
*Goat	1	72	890	604	68
		Primates			
Rhesus monkey	1	60	676.0	16.0	2.5
	2	61	333.0	7.9	2.4
	3	62	312.0	10.8	3.5
	4	92	260.8	8.9	3.4
	5	122	53.7	gone†	
	6	151	48.8	" †	
	7	183	241.8	0 ‡	
	8	183	240.1	14.4	6.0
Cebus monkey	1	29	79.6	5.6	6.7
	2	30	125.5	0 ‡	
	3	31	204.4	25.8	12.6
	4	100	139.1	0 ‡	
	5	145	216.2	0	
	6	145	220.6	8.5	3.9

* Data from Lutton and Large(2).
† Small stones may have been passed.
‡ Stone obstructed cystic duct.

Sjoval(7). Haslewood and Wootton isolated cholic acid from Rhesus monkey bile(8). However, in the present experiment, in addition to cholic acid, paper chromatogram showed presence of chenodeoxycholic and deoxycholic acids in Rhesus monkey bile. In Cebus monkey bile, cholic and chenodeoxycholic acids were present. The presence of a small but definite spot, which has an Rf value between cholic and chenodeoxycholic acids, has been demonstrated on paper chromatogram of bile acids from both kinds of monkeys studied.

Discussion. Although many studies on cholesterol metabolism have been carried out using usual experimental animals, it is not usually possible to assume that these hold in man(9). Gallstones which consist primarily of cholesterol are a definite case in point. We have never found gallstones rich in cholesterol in animals other than man, and furthermore, human gallstones are dissolved when placed in the gallbladder of other experimental ani-

TABLE II. Comparison of Major Constituents of Bile from Man, Monkey and Dog.

	No. of samples	Cholesterol, mg %	Phospholipids, mg/ml	Bile acids			
				Cholic	Deoxycholic	Chenodeoxycholic	Total bile acid
				mg/ml			
Human							
Hepatic bile	3	113.8 ± 22.9	8.11 ± 1.43	14.1 ± 1.25	1.19 ± .13	2.9 ± .25	18.2 ± 1.84
Gallbladder bile pooled		690.0		26.9	5.6	16.0	48.5
Cebus monkey							
Hepatic bile	3	45.1 ± 23.4	17.3 ± 1.60	3.55 ± 3.56	.15 ± .087*	.58 ± .086	4.28 ± 3.71
Gallbladder bile	7	207.0 ±106.3	26.07 ±15.1	36.6 ±16.3	2.28 ± .60	12.26 ±6.70	51.24 ±20.6
Rhesus monkey							
Hepatic bile	2	49.8 ± 8.2	4.55 ± .67	4.8 ± 1.4	1.6 ± .25	1.1 ± .0	7.4 ± 1.6
Gallbladder bile	2	202.4 ± 82.6	13.20 ± 1.70	28.6 ± 1.2	8.6 ± .4	5.0 ± .2	42.2 ± 1.4
Dog							
Hepatic bile	4	26.3 ± 11.8	13.96 ± 4.27	21.6 ± 6.28	3.29 ±2.22	2.05 ± .32	26.89 ± 8.63
Gallbladder bile pooled		70.0		6.75	15.2	6.4	89.1

* Value for deoxycholic acid is influenced by presence of large amounts of other bile acids(5). All values expressed as mean ± S.D.

mals(1,2), as bile of usual experimental animals can hold more cholesterol in solution than is normally present. On the other hand, human bile is more saturated with cholesterol (3). In our experiment we found that human gallstones are much less soluble in monkeys than in usual experimental animals (Table I), suggesting that monkey bile is more saturated with cholesterol.

There have been few reports indicating incidence of gallstones in monkeys(10). However, it is doubtful that gallstones in monkeys are identical to those of man, which contain a large amount of cholesterol. Lapin and Yakovlava(11) stated they found coagulated gall in the form of cholesterin pigmented clots or cholesterol sand. Fox(12) reported finding pigmented gallstones in the pig-tailed monkey (*Macacus nemestrinus*) but did not analyze the stone. Thudichum(13) stated that gallstones of monkeys are similar to those of man, but he also included pigs, which are now known to have stones of lithocholic acid (14).

Summary. 1. Human gallstones placed in monkey gallbladders are less soluble than those placed in other experimental animals. 2. The major constituents in monkey bile are compared with those in human and dog bile.

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Routine Development of Permanent Strains of Fibroblasts from Bone Marrow of Adult Rabbits. (25732)

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Permanent cell lines of fibroblasts have been established *in vitro* from fetal and adult rabbit tissues, but development of these lines was accomplished only after many failures (1). During studies designed to develop methods for continuous growth and maturation of rabbit bone marrow cells *in vitro* permanent strains of rapidly multiplying fibroblasts could be routinely established from explants of adult rabbit bone marrow. This report describes the relatively simple method used for routine development of permanent strains of fibroblasts from rabbit bone marrow and some growth and morphologic characteristics by these cell lines.

Material and methods. Six-week to one-year-old rabbits were anesthetized with nembutal and a femur was resected. The femur was grasped between 2 large hemostats and cracked with a twisting motion and marrow was carefully removed with No. 11 Bard Parker blade attached to No. 7 holder and placed in a 50 mm Petri dish in 5 ml of culture medium composed of 1 part calf serum, 2 parts medium 199(2) and 0.1% yeast extract (Difco). 400 Units of penicillin/ml and 80 μg/ml of streptomycin were routinely