sion). Total activity appearing in the perfusion solution was calculated from Table I: the LPL activity obtained from the perfusion solution was about 1.5 times that obtained from the ammonium extractable LPL of heart.

Summary. With a perfused, isolated rabbit heart LPL activity appeared in the perfusion solution of bicarbonate buffer in the presence of 5 mg % heparin and 5 to 10% serum. However, under the same conditions, LPL did not appear with phosphate buffer. The LPL produced was inhibited by protamine sulfate, $CaCl_2$ and NaCl. Optimum pH of enzymatic activity was at pH 8.2, and 80% of the activity was lost by heating at 50°C for 2 min and 70°C for 30 sec. After perfusion, LPL activity in the heart disappeared. Total activity appearing in the circulating solution was nearly equal to total activity of heart tissue which was extractable with ammonium buffer solution.

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Conversion of Mevalonic Acid-2-C^{14} to Biliary Cholesterol and Cholanic Acids in the Guinea Pig.* (26777)

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Since mevalonic acid was discovered(1) numerous studies have been conducted to show the conversion *in vitro* of this compound to cholesterol(2,3). Important intermediates in the metabolic pathway appear to be phosphomevalonic acids, pyrophosphates of isopentenyl and dimethylallyl alcohol, geraniol, farnesol and nerolidol, as well as squalene and lanosterol(3). Cholesterol, in turn, is the major precursor of bile acids. In comparison to many studies on conversion of mevalonic acid to cholesterol *in vitro,* few investigations have been reported on *in vivo* conversion to cholesterol(4) or to bile acids. The present study concerns *in vivo* conversion of mevalonic acid-2-C^{14} to labeled cholesterol and bile acids in guinea pig.

Materials ad methods. Each of 25 guinea pigs of either sex, weighing 140-180 g, received, intravenously, 3.26 μc (0.772 mg) mevalonic acid-2-C^{14} in 0.10 ml 0.15 molar NaCl. Four hours later, all animals were sacrificed and the contents of their gall bladders pooled. Bile acids and cholesterol were then isolated, identified and the C^{14} content determined by the following procedures.

The 4.20 ml of pooled bile were hydrolyzed in 1 N NaOH for 5 hours at 110°C, extracted with ethyl ether and further analyzed on a column of hydrophobic kieselguhr using chloroform:heptane (9:1) as stationary phase and 58% aqueous methanol as mobile phase(5). Aliquots of the original sample of pooled biles, of the ether extract, and of the fractions of the effluent from the column were applied to aluminum planchets and their C^{14} content determined by a windowless flow counter with accumulation of sufficient counts for 1% standard error.

Results. The pooled bile contained 2.2% of injected C^{14}. Of this activity, 99.3% was recovered in ether extract after hydrolysis. Most of the C^{14} applied to the column was found in the fractions having the mobility of 3α, 7α-dihydroxycholanic (59.7%) and 3α-hydroxy, 7-ketocholanic acid (28.5%). After collection of 150 ml of effluent, the column was eluted with $CHCl_3$, and 11.8% of C^{14}

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was recovered, representing the non-mobile "neutral sterols."

The 2 labeled bile acids were mixed with corresponding unlabeled acids, obtained from guinea pig bile and identified by column (5) and paper chromatography(6), infra red spectra, spectra in conc. H_2SO_4, specific rotation and melting point. No depression of melting points was observed and C^{14}/mg remained constant after several recrystallizations. To identify the sterols in $CHCl_3$ eluate of the column, digitonin treatment(7) was performed. After the digitonide was split with pyridine, 91% of C^{14} (10.7% of total activity applied to the column) was recovered. This labeled material was mixed with authentic unlabeled cholesterol and brominized(8). Following splitting of dibromo compound, all activity was recovered as cholesterol. C^{14}/mg remained constant after several recrystallizations and the melting point was not depressed.

Comments. The results of the present study demonstrated that labeled cholesterol and bile acids appeared in bile of the guinea pig soon after intravenous administration of mevalonic acid-2-C^{14}. Gould and Popják(4) recovered approximately half of the injected C^{14} from the urine of the rats within 4 hours following intraperitoneal administration of mevalonic lactone-2-C^{14}, thus supporting the suggestion of Tavormina and coworkers(2) that only one enantiomorph of mevalonic acid is utilized for biosynthesis of cholesterol, the unnatural (−)-isomer being excreted in the urine(9). Urinary recovery of 48.7% of C^{14} after intravenous injection of mevalonic acid-2-C^{14} in guinea pig confirms the above observations in the rat.

Since conversion of cholesterol to bile acids has been established in many animals as well as the guinea pig, it would appear that mevalonic acid may have been converted first to cholesterol and thence to bile acids. According to the Woodward-Bloch proposal for squalene cyclization to sterol(10) and the positions of incorporation of C^{14} from mevalonic acid-2-C^{14} into cholesterol(3), one C^{14} atom per molecule or 20% of the isotope is lost in conversion of cholesterol to bile acids. Guinea pigs with continuous enterohepatic cycling for 4 hours after intravenous administration of cholesterol-4-C^{14} had 1.7% of injected C^{14} per ml of bile(11). In the present study, after a comparable period of enterohepatic cycling, the guinea pigs excreted in bile 4.4% of C^{14} from mevalonic acid not excreted in urine. Adjusting this to the 4.2 volume and by the 20% loss in conversion of cholesterol to bile acids, one arrives at a value of approximately 1.3% injected C^{14}/ml bile. The similar values for biliary excretion following mevalonic acid and cholesterol suggest that most of the mevalonic acid not excreted in urine entered the cholesterol pool as such. This ratio of conversion of mevalonic acid-2-C^{14} to cholesterol is similar to the observation in the mouse by Gould and Popják(4).

The 2 labeled bile acids isolated in this study, 3α, 7α-dihydroxycholanic (chenodeoxycholic) and 3α-hydroxy, 7-ketocholanic (7-ketolithocholic) acid, the only bile acids present in the bile of immature guinea pigs (12), appear normally in approximately the same ratio as observed in the present investigation.

Summary. After intravenous administration of mevalonic acid-2-C^{14} the labeled constituents of guinea pig bile were cholesterol, 3α, 7α-dihydroxycholanic (chenodeoxycholic) and 3α-hydroxy, 7-ketocholanic (7-ketolithocholic) acid. The relative amount of C^{14} in these compounds suggested that most of the C^{14} not excreted in urine entered the cholesterol pool as such.

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Effects of Controlled Acute Hemorrhage on Myocardial Contractile Force. (26778)

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The concept that cardiogenic factors are contributory to death from shock has been well documented since the early observations of Werle, Cosby and Wiggers(1). These investigators showed that pulsus alternans, progressive bradycardia, and an elevated right atrial pressure are terminal features of hemorrhagic shock. Pathologic study of dog hearts following hemorrhagic shock(2) has suggested that myocardial ischemia is the underlying cause of the cardiac depression. Further support is lent to this view by studies of coronary blood flow and myocardial oxygen consumption(3,4,5,6) which demonstrated that flow is decreased in spite of coronary vasodilatation and that myocardial oxygen consumption is reduced even though the extraction of oxygen from coronary arterial blood is increased. That a net decrease in cardiac efficiency is associated with shock has been generally agreed upon, but some investigators feel that the observed reduction in coronary flow is insufficient evidence that cardiac failure contributes to the progression of hemorrhagic shock and have suggested that other properties of the myocardium be evaluated to elucidate further the mechanics of adjustment to the shock state(7). In the present study myocardial contractile force was measured during acute hemorrhage and onset and extent of myocardial depression assessed by this means.

Methods. Adult mongrel dogs weighing 9-14 kg and apparently in good health were anesthetized with thiopental (30 mg/kg) and respiration was maintained through a cuffed endotracheal tube attached to a piston-type respirator. Central aortic pressure was obtained by cannulation of one femoral artery and the opposite femoral artery was cannulated and connected to an elevated calibrated reservoir. A Walton-Brodie strain gauge arch(8) was sutured to the anterior surface of the right ventricle after the right chest had been opened. The segment of myocardium between the points of attachment of the arch was stretched by at least 50% of its initial length so that any change in fiber length would not affect the measurement of contractile force(9). Pressures measured by means of Statham transducers were recorded continuously, along with myocardial contractile force, on a direct-writing polygraph. The animals were given heparin intravenously (2 mg/kg) and after a control observation had been made they were allowed to bleed into the reservoir at a steady rate. Eight dogs were bled at a rate which lowered mean aortic pressure to 50 mm Hg within 2 minutes; 4 dogs were bled to this level of pressure in 5 minutes and another 4 dogs were bled to this pressure in 10 minutes. Blood pressure was maintained at or below 50 mm Hg by continued withdrawal of blood as necessary during the period of observation and the animals were then sacrificed.

An additional group of 4 dogs was studied utilizing total right heart bypass. Cannulae were inserted into the superior and inferior vena cavae and a third cannula was inserted into the right ventricle to return coronary blood and provide total ventricular decompression. The venous return from these cannulae was collected in a reservoir and re-