JOURNAL OF SURGICAL RESEARCH 28, 1–7 (1980)

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The Effect of Portacaval Shunt upon Hepatic Cholesterol Synthesis and Cyclic AMP in Dogs and Baboons[1,2]

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Submitted for publication June 26, 1979

Hepatic cholesterol synthesis, hepatic cyclic AMP, and portal and peripheral insulin and glucagon levels were investigated in nine dogs and three baboons after complete portacaval shunt. Cholesterol synthesis as measured with acetate incorporation was reduced in both species. Hepatic cyclic AMP increased in dogs. Changes in portal and systemic insulin were inconsistent, but hyperglucagonemia occurred regularly. Diminished hepatic cholesterol synthesis is apparently one factor, although probably not the only one, in the antilipidemic effect of portacaval shunt. This altered cholesterol metabolism may be due to a change in the hormonal environment of the liver caused by portal diversion.

INTRODUCTION

In rats [17, 25], dogs [1–13, 19, 22, 33, 36], pigs [8, 9], baboons [28, 33], and humans [4, 34, 35] completely diverting portacaval shunt causes a decrease in serum cholesterol. The mechanism of this antilipidemic effect is, however, a matter of dispute. We reported earlier that there was a diminution of hepatic cholesterol synthesis [33] but this effect in dogs has been denied by Coyle [11–13] and Guzman [19] and their associates. In this communication, the role of altered hepatic cholesterol synthesis in lowering serum cholesterol has been reexamined in dogs and baboons subjected to portal diversion.

[1] The advice and technical help of Oscar K. Reiss, Ph.D., and Mrs. Ellie Groeneboer is gratefully acknowledged.

[2] The work was supported by Research Grants MRIS 8118-01 and 7227-01 from the Veterans Administration; by Grants AM-17260 and AM-07772 from the National Institutes of Health; and by Grants RR-00051 and RR-00069 from the General Clinical Research Centers Program of the Division of Research Resources, National Institutes of Health.

MATERIALS AND METHODS

Experimental preparation. Nine dogs weighing 12.7 to 31.8 kg had side-to-side portacaval anastomoses with high ligation of the portal vein creating a functional complete end-to-end shunt [33]. Animals were studied and then sacrificed 1 month after operation.

Three female baboons weighing 11.8 to 17.5 kg had end-to-side portacaval shunts. Three months later, a liver biopsy and blood sample were taken and the animals were sacrificed.

Dogs were anesthetized with ketamine hydrochloride or phenylcyclidine hydrochloride, and succinylcholine chloride for both operation and sacrifice. Phenycyclidine hydrochloride was used as anesthesia for both procedures in baboons.

Cholesterol determination. All serum and tissue samples were obtained between 9:00 and 10:00 AM after a 14-hr fast. For both serum and liver extracts, cholesterol concentration was determined by the method of Zlatkis and his associates [39].

Cholesterol synthesis. A specimen was excised from the liver, rapidly cooled in ice-

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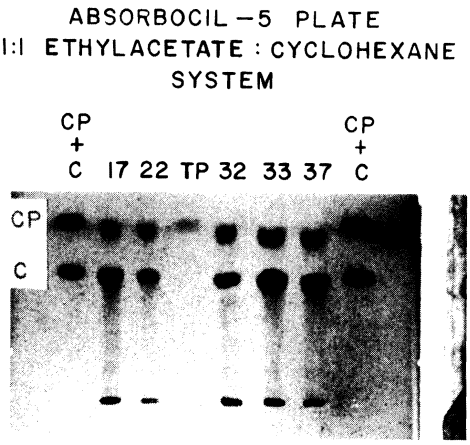

FIG. 1. Thin-layer chromatography showing separation of raw extract (bottom) into cholesterol (middle, C) and cholesterol palmitate (top, CP). TP, CP, and C are controls for cholesterol tripalmitate, cholesterol palmitate, and cholesterol, respectively. Numbered samples are from different dogs.

cold saline, and then sliced. Two hundred milligrams of slices was placed in 15-ml incubation flasks containing 2 ml of Krebs–Hanseleit bicarbonate buffer with a pH of 7.4, 30 μmole of sodium acetate, and 20 μCi of [2-^{14}C]acetic acid sodium salt with specific activity of 50 mCi/mmole. After 2 hr of incubation at 37°C, the reaction was terminated by introduction of 2.4 ml of 15% KOH in absolute ethanol under an atmosphere of 95% O_2–CO_2. These samples were then saponified over steam for 2 hr and extracted with petroleum ether to remove nonsaponifiable lipids (three 20-ml extractions).

Petroleum ether extracts from each sample were pooled, dried out, and the residue taken up in 4 ml of twice-distilled chloroform. Cholesterol determinations were made on a digest of this extract by the Lieberman–Burchard reaction [39]. The remaining chloroform extract was used for determining the cholesterol synthesis using two different methods: 1.5-ml samples from three dogs and all baboons were subjected to digitonin purification according to the principle of Sperry [32]; 1-ml samples from all dogs were subjected to thin-layer chromatography in 1:1 ethyl acetate–cyclohexane. With this latter method, using cholesterol and cholesterol palmitate and tripalmitate as controls, we achieved good separation of cholesterol from contaminants (Fig. 1).

With both methods, radioactivity of the extracted cholesterol was determined by liquid scintillation counting using Instagel mixture. The rate of cholesterol synthesis was expressed as disintergrations per minute per 100 μg of cholesterol extract.

Cyclic adenosine 3,5'-monophosphate (cyclic AMP). Cyclic AMP concentration in the liver was analyzed by the radioimmunoassay of Harper and Brooker [20] from a trichloroacetic acid extract that had been passed through a Dowex 50 column (700–400 mesh).

To determine the rate of formation of liver cyclic AMP, three normal dogs and five dogs 1 month after portacaval shunt were subjected to an aminophylline infusion test under anesthesia. As described by Robison and his group [20], the methylxanthines in appropriate dosages are essentially complete inhibitors of cyclic AMP phosphodiesterase. Butcher and Sutherland [7] and Cheung [10] have shown this phosphodiesterase to be the principal enzyme involved in the catabolism of cyclic AMP. Rapid intravenous infusion of animophylline inhibits this enzyme and allows the quantitation of cyclic AMP formation. Infusion over 6 min permits prompt completion of studies before the onset of delayed aminophylline-induced effects, such as changes in insulin levels which might by themselves affect the metabolism of cyclic AMP.

Control biopsy specimens of the right and left lobes were taken for baseline levels. Then, 3 g of aminophylline in 300 ml of 5% dextrose in normal saline solution were infused by way of a peripheral vein at a constant rate over 6 min. Biopsy specimens of 100 to 200 mg in weight were removed from both sides of the liver at 2, 4, and 6 min after the infusion was begun, taking care to minimize blood loss. These were immediately frozen in liquid nitrogen and

assayed for cyclic AMP as previously described.

Glucagon and insulin determinations. Glucagon and insulin concentrations in both portal vein and inferior vena cava were determined in the laboratory of Dr. R. H. Unger of Dallas, Texas, in eight of the nine dogs. Blood specimens were drawn before portacaval shunt and again just prior to sacrifice. Insulin analysis was by the immunoassay of Herbert and his associates [21]. Glucagon was determined by the radioimmunoassay of Faloona and Unger [18]. The primary pancreatic glucagon measured with this technique has a molecular weight of 3500, although other larger moieties have some activity.

RESULTS

Clinical behavior. Dogs lost weight from 19.1 ± 5.3 to 12.4 ± 2.4 (SD) kg during the 1 month observation period. None developed encephalopathy. The baboons weighed 11.8, 17.5, and 13.6 kg at the time of portacaval shunt. During the 3 months, their weights fell to 9.5, 13.2, and 9.7 kg. Although all had hair loss, none had obvious encephalopathy.

At sacrifice, all shunts were explored and found to be patent.

Serum cholesterol. In dogs, serum cholesterol fell from 160 ± 54 to 131 ± 39 mg/100 ml. In baboons, cholesterol decreased from 129 ± 27 to 83 ± 12 mg/100 ml. Serum cholesterol decreased in seven of nine dogs and in all three baboons.

Liver cholesterol. Liver cholesterol increased significantly following portacaval shunt in dogs (Table 1). No significant change in liver cholesterol was demonstrated in the baboons (Table 1).

Cholesterol synthesis. In dogs, hepatic cholesterol synthesis decreased to a significant degree following portacaval shunt (Table 2). Rates of cholesterol synthesis obtained by the digitonin method and by thin-layer chromatography in dogs were comparable and demonstrated decreases. Highly significant decreases were found in the baboons using the digitonin method. Because liver cholesterol had increased in some animals, changes in the absolute role of cholesterol synthesis were also computed (Table 2).

TABLE 1

HEPATIC CHOLESTEROL IN DOG AND BABOON BEFORE AND AFTER PORTACAVAL SHUNT

Animals	Cholesterol (μg/g wet liver) Before	After
Dogs		
7	1520	2291
8	1580	1855
9	1596	2006
16	2130	2693
17	—	—
22	1205	2149
32	2296	2410
33	2095	1268
37	2105	2965
M ± SD	1886 ± 363	2267 ± 385
	$P^* < 0.001$	
Baboons		
1	3546	3252
2	3183	4911
4	3684	3564
M ± SD	3471 ± 211	4075 ± 595
	P^* NS	

* For paired data by Student's *t* test.

Hormonal studies. After portacaval shunt, insulin levels in the portal vein decreased from 78 ± 76 to 60 ± 58 μU/ml. Insulin in the vena cava increased from 21 ± 20 to 31 ± 25 μU/ml. Neither change was significant. On the other hand, glucagon concentration increased significantly from 151 ± 76 to 328 ± 196 pg/ml in portal vein and from 59 ± 41 to 183 ± 91 pg/ml in vena cava. In two dogs in whom cholesterol synthesis decreased (dogs 16–22 in Table 2), portal venous glucagon concentrations did not increase after portacaval shunt.

Cyclic AMP. Cyclic AMP increased significantly in dog liver after portacaval shunt (Table 3). This increase was not documented in baboon liver.

Aminophylline test. The rate of cyclic AMP formation as determined by the amino-

TABLE 2

HEPATIC CHOLESTEROL SYNTHESIS IN DOG AND BABOON LIVER BEFORE AND AFTER PORTACAVAL SHUNT

	Digitonin–thin-layer chromatography				Digitonin			
	Cholesterol (dpm/100 μg of hepatic cholesterol)				Cholesterol (dpm/100 μg of hepatic cholesterol)			
			Percentage decrease				Percentage decrease	
Animals	Before	After	From raw data	Adjusted for increased hepatic cholesterol	Before	After	From raw data	Adjusted for increased hepatic cholesterol
Dogs								
7	3291	5737	—	—				
8	5839	3494	40	28				
9	1802	495	72	66				
16	3565	496	86	82	2954	286	90	88
17	5031	2269	54	*	4500	2097	53	*
22	2932	896	68	45	3962	1205	69	64
32	2380	1220	49	46				
33	3324	5002	—	—				
37	1751	674	61	46				
M ± SD	3323 ± 1375	2254 ± 2025			3805 ± 640	1195 ± 738		
	$P^{**} < 0.001$				$P^{**} < 0.001$			
1					1682	140	91	91
2					1237	415	66	48
4					966	461	52	54
M ± SD					1295 ± 235	338 ± 141		
					$P^{**} < 0.01$			

* Hepatic cholesterol data not available.
** Paired data compared by Student's *t* test.

phylline test in dogs (Fig. 2) was not significantly altered by portacaval shunting. Higher baseline levels of hepatic cyclic AMP were again noted in many of the dogs that had undergone portacaval shunting.

DISCUSSION

Although it has been well established that portal diversion lowers serum cholesterol in several species, the mechanism of this effect has been a matter of dispute. Our studies [33] in the dog, as well as investigations in the rat [17, 23], pig [8, 9], and human [4] have suggested that a reduction in cholesterol or low-density lipoproteins (LDL) synthesis is responsible at least in part for the cholesterol falls. However, studies by Coyle and his associates have not been confirmatory [11–13, 19].

Our present investigations have shown once more that hepatic cholesterol synthesis is reduced following portacaval shunt in both dogs and baboons. For the canine studies, two techniques for measuring cholesterol synthesis were used which differed in the way in which cholesterol was separated from other lipids. Both measured labeled acetate incorporation. The results were almost identical. In calculating synthesis rates, the increased hepatic cholesterol content in the dogs was taken into account. Although the adjustment lessened the falls in cholesterol synthesis, the reductions were still substantial and significant. Ultimately, the calculations of hepatic cholesterol synthesis were per aliquots of hepatic tissue. Portacaval shunt in dogs [23] and baboons [28, 33] has been shown invariably to produce atrophy with a striking reduction of total hepatic mass. Thus, the expression of results undoubtedly was a striking understatement of the actual reduction in total hepatic cholesterol synthesis.

The exact mechanism by which portal diversion reduced cholesterol synthesis remains open to speculation. Our earlier experiments in dogs [33] suggested that one

reason might be deprivation of the liver of endogenous insulin resulting in a moderately severe liver injury. Alternatively, Eaton [16] has proposed that changes in glucagon metabolism might be important in causing the antilipidemic effect. Portacaval shunting has also been demonstrated to cause increases in serum glucagon concentration [26, 30, 31]. Glucagon in pharmacologic [27] or physiologic [37] doses has been shown to decrease hepatic cholesterol synthesis possibly via an increase in hepatic cyclic adenosine 3′,5′-monophosphate (cyclic AMP). We therefore also measured serum glucagon and hepatic cyclic AMP changes following portal diversion in an attempt to correlate them with cholesterol synthesis rates.

In the present study, no further information about insulin was adduced. The peripheral hyperinsulinemia that has been described after portacaval shunt [3, 14, 15] was not consistently seen. However, increased glucagon levels in the systemic venous blood were regularly observed in confirmation of earlier reports [26, 30, 31]. As might be expected with hyperglucagonemia in combination with a bypassing of insulin around the liver, the hepatic cyclic AMP was consistently elevated in the dogs, but, surprisingly, not in the baboons. The canine increases occurred in spite of the fact that cyclic AMP production as measured by the aminophylline test was not strikingly increased. In addition, cyclic AMP increased despite the known decrease in responsiveness of adenyl cyclase receptors to glucagon stimulation in hyperglucagonemic states [38]. Elevation in cyclic AMP has been described in inhibiting cholesterol synthesis [2, 5, 6].

These experiments have added weight to the contention that reduced hepatic cholesterol synthesis is at least part of the reason for the antilipidemic effect of the portacaval shunt, although it seems unlikely that this is the sole explanation. Ahrens [1] has speculated than an increased loss of bile acids with an obligatory drain on the cholesterol pool is a major contributing factor. Other possibilities have been suggested by Kuo *et al.* [24] and Coyle *et al.* [12].

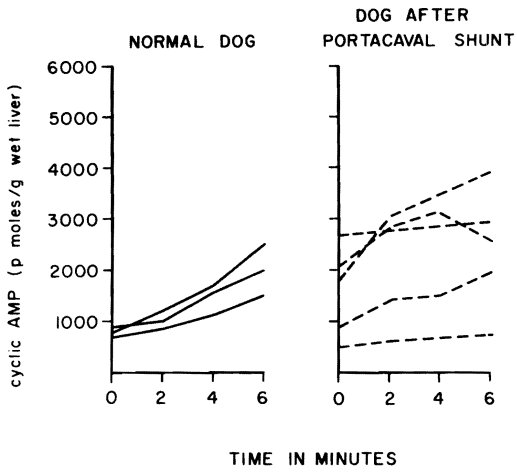

FIG. 2. Aminophylline test results showing cyclic AMP generated at 2, 4, and 6 min for three normal and five shunted dogs.

TABLE 3

CYCLIC AMP CONCENTRATION IN DOG AND BABOON LIVER BEFORE AND AFTER PORTACAVAL SHUNT

Animals	cAMP (pmol/g of wet liver)	
	Before	After
Dogs		
7	778	947
8	565	1940
9	1141	1949
16	1590	2798
17	1147	2085
22	964	518
32	757	1232
33	730	1345
37	653	1432
M ± SD	925 ± 322	1583 ± 682
	$P^* < 0.01$	
Baboons		
1	920	876
2	—	618
4	763	695
M ± SD	841 ± 78	729 ± 108
	P^* NS	

* Compared to preoperative value using Student's *t* test for paired data.

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