# Effect of Common Bile Duct Ligation on Lipoprotein Lipase Response in Dogs.*†‡ (24981)

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*Decreased* heparin-activated lipoprotein lipase (clearing factor) response has recently been reported in patients with obstructive jaundice(1,2,3). The etiology of this jaundice, however, varied greatly, being both intra- and extra-hepatic in origin, and subjects manifested varying degrees of inanition, acidosis or electrolyte imbalance. Consequently, to determine more precisely time course of events in obstructive jaundice, we completely ligated the common bile duct in normal dogs.

*Material and methods. Subjects.* In preliminary experiment, a female mongrel dog (Q) weighing 7.5 kg was subjected to complete ligation of common bile duct under pentobarbital sodium anesthesia. Subsequently, 6 female mongrel dogs (A-F), weighing 12 to 16 kg were placed on stock laboratory diets. After 3 week control period, complete ligation of common bile duct was performed on all 6 dogs under pentobarbital sodium anesthesia. *Lipoprotein lipase response.* Standardized *in vitro* method previously reported (3,4,5) for determining clearing factor or lipoprotein lipase concentration was employed. Control and 8 minute post-heparin blood samples were obtained from each dog at weekly intervals after overnight fast (at least 12 hours). Plasma was extracted by centrifugation at 0°C and frozen until used. *Evaluation of hepatic status.* At weekly intervals during 3 week control period, evaluation of hepatic status was performed on all 6 dogs. This included sulfobromphthalein (BSP) excretion, total and esterified cholesterol, serum bilirubin, icterus index, total proteins, albumin/globulin ratio, cephalin flocculation, thymol turbidity, gamma globulin turbidity, and alkaline phosphatase. Total serum lipids and lipid phosphorus were also determined on aliquots of control and post-heparin plasma samples.

*Results.* Table I demonstrates the effect of common bile duct ligation on lipoprotein lipase response in all dogs. Lipoprotein lipase response was *markedly decreased* in 4 out of 7 dogs one week after ligation of common bile duct, in fifth dog, 2 weeks, and in sixth and seventh dogs, 5 weeks after ligation. All 5 dogs who lived 4 weeks or more after surgery demonstrated *subsequent marked increase* in lipoprotein lipase response 4 to 10 weeks after ligation of common bile duct.

One dog (C) died one week postoperatively, death unrelated to experiment. A second dog (D) died 3½ weeks postoperatively with evidence of gross internal hemorrhage. Both dogs had markedly decreased lipoprotein lipase responses prior to death.

Lipoprotein lipase response was *generally inversely proportional* to total serum lipid, total serum cholesterol and lipid phosphorus levels. In Dog A, mean control lipoprotein lipase was 1.60, while mean control total lipids were 820 mg%. One week after common bile duct ligation, lipoprotein lipase response was markedly decreased to 0.76, while total serum lipids had doubled to 1820 mg %. Four weeks postoperatively, lipoprotein lipase response became distinctly elevated to 4.20, with abrupt drop in total serum lipids below control values to 505 mg %. Four days later this dog died. Postmortem findings included perforation of greatly dilated common bile duct with bile peritonitis and hemorrhage. The liver grossly demonstrated greatly dilated bile canaliculi.

In 4 dogs, B, E, F, and Q, *sustained* hyperlipemia and hypercholesterolemia were present for 2, 3, 5, and 5 weeks, respectively, be-

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TABLE I. Effect of Common Bile Duct Ligation on Lipoprotein Lipase Response in Dogs.

| Date samples drawn | Weeks after samples drawn | *k* † Dog Q | Dog A | Dog B | Dog C | Dog D | Dog E | Dog F |
|---|---|---|---|---|---|---|---|---|
| 12/ 8/58 | 0 | Ligation | | | | | | |
| 15 | 1 | 1.26 | | | | | | |
| 22 | 2 | | | | | | | |
| 29 | 3 | | 1.63 | 1.51 | | | | |
| 30 | | | | | 1.72 | 2.24 | 2.47 | 1.39 |
| 1/ 5/59 | 4 | * | | | 1.35 | 4.50 | | |
| 8 | | 1.25 | 1.94 | | | | 1.55 | 1.44 |
| 12 | 5 | | 1.33 | 2.31 | 1.97 | 1.83 | | |
| 15 | | .48 | | | | | 1.22 | 1.34 |
| 19 | 6 | | Lig.‡ | Lig. | Lig. | Lig. | Lig. | Lig. |
| 22 | | .73 | | | | | | |
| 26 | 7 | | .76 | 1.86 | .21 | .82 | | |
| 29 | | .72 | | | Exp. | | | 1.47 |
| 2/ 2 | 8 | | .20 | .24 | | .24 | | |
| 5 | | .64 | | | | | 2.06 | 2.07 |
| 9 | 9 | | .62 | .55 | | .14 | | |
| 12 | | 6.48 | | | | | .51 | 2.23 |
| 13 | | | | | | Exp. | | |
| 16 | 10 | 2.00 | | 1.44 | | | | |
| 19 | | | 4.20 | | | | .21 | 2.31 |
| 23 | 11 | 1.98 | Exp. | 4.80 | | | | |
| 24 | | Exp.‡ | | | | | | |
| 26 | | | | | | | .46 | .21 |
| 3/ 2 | 12 | | | 2.39 | | | .21 | .23 |
| 9 | 13 | | | 3.96 | | | .52 | 2.32 |
| 16 | 14 | | | 7.05 | | | | 1.79 |
| 21 | | | | Exp. | | | | |
| 23 | 15 | | | | | | .42 | 1.08 |
| 25 | | | | | | | | Exp. |
| 30 | 16 | | | | | | 1.80 | |
| 4/ 5 | 17 | | | | | | 2.24 | |
| 7 | | | | | | | Exp. | |

* Control period before common bile duct ligation.

† *k* is rate of decrease of optical density/hr. This is directly proportional to lipoprotein lipase (clearing factor) concentration in standardized *in vitro* assay method used(3,4,5).

‡ Exp. = Expired; Lig. = Ligation.

fore initial decrease in lipoprotein lipase response was observed. In 2 dogs, B and Q, total serum lipids reached levels as high as 2720 and 2880 mg %, respectively, serum total cholesterol being 430 and 422 mg %. These levels were accompanied by essentially *normal* lipoprotein lipase responses of 1.86 and 1.25, respectively. However, a significant ($P<.01$) negative regression of lipoprotein lipase response with increasing total serum lipids was found when all dogs were considered. Hyperbilirubinemia, *per se,* did not appear to influence lipoprotein lipase response. Perhaps the most remarkable elevation subsequent to biliary ligation occurred in serum alkaline phosphatase. In Dog A this reached 102.6 Bodansky units 3 weeks after ligation. Dog E rose to 126.3 units *one* week after ligation and 141.4 units 2 weeks after ligation. This observation, while not unique, is nevertheless quite striking when compared to humans with biliary obstruction. Lipid phosphorus rose with total lipids and cholesterol.

Percentage of cholesterol esters was variable, rising after biliary ligation in some instances, and dropping markedly preterminally in Dogs A, B, E, and F. Cholesterol/lipid phosphorus ratio demonstrated no trend. Neither of these parameters was related to lipoprotein lipase response. Total protein, A/G ratio, cephalin flocculation, and thymol turbidity were not very useful as indices of hepatic function or functional impairment. All dogs demonstrated $3^+$ and $4^+$ cephalin flocculation during *control* period, and in A, C, and E there was inversion of the A/G ratio during *control* period. This is generally found to occur in normal dogs. Forty-five minute BSP retention, however, was 4% or less during control period in all 6 dogs.

A pre-terminal increase in gamma globulin turbidity was probably concomitant with hepatic parenchymal cell damage. Fasting blood sugar levels were variable, Dog A demonstrating hyperglycemia (118 mg %) pre-terminally, and Dogs Q, E, and F demonstrating hypoglycemia (36, 24, and 45 mg %, respectively). *Decrease* in total lipids, total and esterified cholesterol, and lipid phosphorus generally accompanied the subsequent *increase* in lipoprotein lipase response, presumably reflecting severe hepatic parenchymal cell damage and biliary cirrhosis. Although prothrombin time, determined at intervals, was normal (10 to 12 sec.), some evidence of hemorrhage was generally present at postmortem.

*Discussion.* Hepatic perfusion experiments in rats(6), dogs and man(7) demonstrated inactivation or attenuation of lipoprotein lipase by the *normal* liver. The *markedly decreased* lipoprotein lipase response previously reported in patients with obstructive jaundice has been confirmed in dogs in this experiment. It is suggested that this decreased response is a reflection of abnormal lipid and lipoprotein lipase metabolism. Deficiency of lipoprotein lipase *per se* does not seem likely in view of the *distinctly increased* lipoprotein lipase response occurring subsequently. It is suggested that this increased response may be associated with development of biliary cirrhosis. This was grossly observed in liver of Dog E at postmortem. Microscopic examination of livers of these dogs will be reported. *Increased* lipoprotein lipase response in Laennec's cirrhosis has been previously reported(1,2,3,5), and we have recently reported *increased* response in post-necrotic cirrhosis(8,9). Partial hepatectomy in rats(10), carbon tetrachloride-induced hepatic necrosis in dogs(7) and in rats(10), and patients with Laennec's cirrhosis(7) demonstrated *increased* lipoprotein lipase response in hepatic perfusion studies. It is therefore hypothesized that *increased* lipoprotein lipase response observed subsequent to *decreased* response in dogs after common bile duct ligation represents loss of functional hepatic parenchymal tissue, chronic cholestasis resulting in *chronic* hepatic parenchymal cell damage leading to biliary cirrhosis.

Other possible pathogenetic mechanisms for *decreased* lipoprotein lipase response after common bile duct ligation include abnormalities in lipoproteins *per se,* formation of inhibitor(s) to lipoprotein lipase, or acceleration of *in vivo* inhibition or inactivation of lipoprotein lipase system by the liver. It is conceivable that chronic complete cholestasis may stimulate or activate hepatic parenchymal cells to *increase* their rate of inactivation of lipoprotein lipase. While elevation of serum lipids follows closely upon ligation of common bile duct, the fact that *decrease* in lipoprotein lipase response *lagged* hyperlipemia by as much as 5 weeks in 2 of the 7 dogs suggests that 2 *different* (but perhaps related) mechanisms are involved. Possible factors are being investigated.

*Summary.* 1. *Decreased* lipoprotein lipase response in obstructive jaundice in man has been confirmed by complete ligation of the common bile duct in dogs. 2. Subsequent *increased* lipoprotein lipase response in these dogs after chronic complete cholestasis of 4 to 10 weeks is attributed to development of biliary cirrhosis. 3. While lipoprotein lipase response was *generally inversely proportional* to total serum lipids, cholesterol, and lipid phosphorus levels, this relationship was by no means absolute. 4. Possible pathogenetic mechanisms for *decreased* lipoprotein lipase response in obstructive jaundice have been discussed. These mechanisms are being investigated.

1. Baker, S. P., Levine, H., Turner, L., Dubin, A., *Circulation,* 1958, v18, 483.

2. ———, *Clinical Research,* 1958, v6, 396.

3. ———, PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 670.

4. Baker, S. P., *Circulation,* 1956, v14, 496.

5. ———, *ibid.,* 1957, v15, 889.

6. Jeffries, G. H., *Quart. J. Exp. Physiol.,* (London), 1954, v39, 261.

7. Connor, W. E., Eckstein, J. W., *Circulation,* 1958, v18, 483.

8. Presented at Meeting of Chicago Society of Internal Med., March 23, 1959, Chicago, Ill.

9. Baker, S. P., Levine, H., Turner, L., Dubin, A., *Clinical Research,* 1959, v7, 291.

10. Constantinides, P., So, Y., Johnstone, F. R. C., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 262.

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