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Prothrombin clotting time in dogs with obstructive jaundice.

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PROTHROMBIN CLOTTING TIME IN DOGS WITH OBSTRUCTIVE JAUNDICE

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The surgical and medical risks of infection and other pathological processes in and about the liver are greatly increased by the possibility of hepatic insufficiency. One of the most striking features associated with these cases is their tendency to bleed.

This form of abnormal bleeding, which may result in death from persistent hemorrhage, can apparently begin without trauma, but usually starts at operation or during the postoperative period. Such bleeding has been long known but little understood until recent years. It is now known to result from abnormal lowering of the plasma prothrombin level (hypoprothrombinemia). The attainment of this knowledge is an extremely interesting phase in the very recent era of medical progress.

The existence of vitamin K was first suspected from experiments done in 1929 by Dam (1) of Copenhagen. In some studies of lipoid metabolism, newly hatched chicks were placed on a fat-free diet. After several weeks these chicks developed hemorrhages into the skin, mucous membranes and other parts of the body. This work prompted further study, and it was demonstrated that a new fat-soluble vitamin was essential for the prevention of this bleeding tendency. The Danish word for coagulation is spelled with a "k". The vitamin was therefore spoken of as "vitamin k".

Vitamin K can be produced by bacterial action and is therefore present in the lower portion of the intestine, even when the animal is maintained on a diet free of the vitamin. Absorption from the lower

part of the intestine is minimal in the chick, but in mammals absorption does occur and hence mammals rarely show vitamin K deficiency (2) except in cases of faulty absorption. In chicks maintained on a diet free of vitamin K it was found that the prothrombin level was low, and this explained the bleeding.

The importance of prothrombin in cholemic bleeding has been well brought out by Quick (3) who stated:

"It is fairly generally agreed that four agents are required for the clotting of blood:

1. Prothrombin
2. Thromboplastin
3. Calcium
4. Fibrinogen

"The first three interact to form an active enzyme (thrombin) which reacts with fibrinogen, changing it to an insoluble gel (fibrin), which constitutes the clot. The process can be expressed as occurring in two steps:

1. Prothrombin + Thromboplastin + Calcium = Thrombin
2. Fibrinogen + Thrombin = Fibrin

"From these equations one can conclude that a deficiency of any one of the three factors required for the formation of Thrombin or the absence or marked depletion of fibrinogen will cause a retardation or even a complete inhibition of clotting. Satisfactory quantitative methods for calcium and fibrinogen have long been available, and naturally these two substances have been repeatedly investigated in jaundice. In none of these studies has a significant alteration of either calcium or fibrinogen been established."

Until recently no satisfactory methods for determining prothrombin or thromboplastin were available. This lack no doubt accounted for the failure to consider a deficiency of either of these two important factors as the possible cause of hemorrhage in jaundice. Quick (4) succeeded in developing a procedure for determining plasma prothrombin.

Ordinary tests for bleeding and clotting time were found to be prolonged only when there was an extreme prothrombin deficiency. They still gave normal values when the plasma prothrombin level approached the danger zone. These tests, therefore, fail when they are most badly needed.

Greaves and Schmidt (5) showed that in rats with biliary fistulae a prothrombin deficiency develops, thus confirming similar observations of Quick, Stanley-Brown and Bancroft (6) made in human cases of obstructive jaundice, and of Hawkins and Brinkhous (7) in dogs with biliary fistulae. They also found that this deficiency could be relieved by giving bile salts and vitamin K.

The sluggish prothrombin conversion rate in the plasma of man and guinea pig was found to encourage hemorrhage. The explanation of this is that hemostasis depends upon covering the denuded surface promptly with a film of fibrin and platelets. If coagulation is delayed, the coagulating substances are washed away before an effective clot can form.

It was further shown by Warner, Brinkhous, and Smith (8) that when a dog's liver is partially excised or is injured by poisons, the level of plasma prothrombin falls.

The literature failed to reveal, however, the effect of experimental biliary obstruction on the prothrombin clotting time.

My purpose in this paper is to present experimental observations made in studying dogs in which the common bile duct was ligated. Dogs were chosen because they were known to be cooperative and because they would not bleed to death by lowering of the prothrombin plasma content as might be expected in obstruction of the common bile duct. It is known that such animals as guinea pigs simulate man in hemorrhaging at prothrombin levels far above that necessary to cause bleeding in dogs, rabbits or rats.

The nature of prothrombin is still unknown and all methods of measurement so far proposed are open to objection in that they are not a direct determination of prothrombin.

Quick's method (4) for prothrombin determination requires 4.5 c.c. of the blood to be tested, three solutions, the use of a centrifuge, a somewhat lengthy dehydration, a gravimetric procedure and titration. Warner, Brinkhous and Smith (8) developed a two stage technique which is more specific but even more complex. More recently Ziffren, Owen, Hoffman and Smith (9) developed a simple bedside technique which gave results that closely corresponded to those obtained by the two stage method.

Quick's method was first employed but then abandoned in favor of the bedside one after using both together for a trial period on both humans and dogs.

It is known (10) that if the common bile duct is completely obstructed without removing the gall bladder the onset of jaundice is delayed due to the distention of the gall bladder with bile. It was accordingly decided to remove the gall bladder as

well as to doubly ligate the common bile duct.

A series of six dogs were successfully operated upon under aseptic conditions. Morphine sulphate 1/2 gr. was used for preliminary medication and nembutal 1 c.c. (1 gr.) per 5 pounds of body weight was given intraperitoneally as an anaesthetic.

The blood was drawn from the saphenous vein. The thromboplastic solution was prepared each week by cutting the lungs of a recently killed rabbit into small pieces, mashing with mortar and pestle, adding a few cubic centimeters of physiological saline and straining through gauze. This solution was then tested on normal patients blood. If the prothrombin clotting time was below twenty-five seconds, the thromboplastinwas diluted with additional saline and if above sixty seconds it was discarded according to the directions for its use by the originators (9). The solution was then used on normal dogs each time that the blood was timed for clotting on the animals under observation. The results could therefore be given in per cent of normal. The test consists of adding 0.9 c.c. of blood to 0.1 c.c. of thromboplastin and inverting every second or two in a test tube until clotting occurs.

Two to four days after operation each dog showed tinting of the skin and sclera. The stools became acholic and the urine dark and scanty. After a week the animals began to waste and usually developed ascites, especially if much meat was given. Bilirubin increased from a normal of 0.2 or less to around 3.0 mgm. per 100 c.c. The first dog used lived two months after operation and the last seemed hale and hearty when put to death after about three weeks.

PROTHROMBIN CLOTTING TIME IN PERCENT OF NORMAL

DAYS POSTOPERATIVE

DOG	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	28	35	49	53	56	58	59
1.		52		55.5		50					66.6		40		75		42 (4.5)		60		36.6		41.5	40	42	30	33.3	*
2.			50		40 3.8		37.5		36.6													50 #						
3.	75		66.6				40			66.6 (.3)		62.5	55.5			50 (.6)	R	61 (1.8)		46 *								
4.	66.6		62.5		55.5 (.3)		44		30		40 (2.4)	*																
5.	50	33.3 (3.1)	*																									
6.	70	73.3		60	(2.9)	60		44	45		75 (L)	75 (L)	75 (L)		88			75	100 #									

Bilirubin in ( ), normal = .2 mgm. or less per 100 c.c.
* Died
# Put to death
R Reoperation

L - 0.2 or less. Exact amounts between 0 and 0.2 not calculated

In all the dogs the prothrombin fell to 75-30% of normal, and a tendency to bleed developed at the lower plasma prothrombin levels.

The first dog operated on developed slight jaundice on the second day after operation. Marked icterus appeared on the third postoperative day and continued throughout the lifetime of the animal. Ascites developed during the first week and a bloody diarrhea during the fifth. Both continued from time of onset until death. Autopsy on the day of death showed marked icterus. The ligatures (silk) on the bile and cystic ducts were still tightly in place and there was hydrops of the hepatic ducts. There were adhesions between the liver and duodenum and 250 c.c. of straw colored fluid in the abdomen.

The second dog was put to death after four weeks because he had developed distemper. His course and autopsy findings were essentially the same as the first except that he showed no tendency to bleed.

The third animal did not seem to be completely obstructed and was reopened. An accessory bile duct had been overlooked and when ligated there was an increase in bilirubin and prothrombin clotting time.

The fourth dog died at the end of two weeks. It, like the third, was only moderately jaundiced.

The fifth had gross hemorrhage from the mid-jejunum down with many tapeworms found at autopsy. The lowered plasma prothrombin no doubt led to the profuse bleeding from the tapeworm infested intestine and an early demise.

The last dog ran a rather typical course until the

eleventh postoperative day, at which time his prothrombin clotting time which had been about 45% suddenly jumped to 75%. On the following day it was still 75% and the bilirubin was .2 mgm. per 100 c.c. or less. The bilirubin remained normal from there on and the prothrombin clotting time had returned to normal on the day he was put to death. The post-mortem examination showed the ligature on the common bile duct to be rather loose. It was concluded that it must have been tight enough to produce obstruction as long as postoperative edema was present but when that disappeared the jaundice also left.

When each of the first five animals ultimately died or was killed, the tissues , particularly the liver, were deeply jaundiced. The liver was smaller than normal and sclerotic. The paranchymal cells had largely atrophied, owing to the great interference with the blood supply by the greatly distended ducts (hydrohepatosis). The obstructed ducts were found to contain a whitish mucus, the pigment having been reabsorbed.

CONCLUSION:

In dogs with obstructive jaundice the plasma prothrombin level falls and a tendency to bleed develops.

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