

Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 83

JULY, 1953

No. 3

SECTION MEETINGS

DISTRICT OF COLUMBIA

Washington D. C.

June 4, 1953

SOUTHWESTERN

University of Oklahoma Medical School

May 8-9, 1953

Plasma Coagulation Factors in Acute Pancreatitis.* (20377)

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Since thrombosis has been associated with pancreatic diseases(1), particularly with carcinoma of the pancreas(2) some investigations on the blood coagulation mechanism(3-6) in this group of diseases have been made. For some time in this laboratory coagulation components have been determined quantitatively on blood fractions(7). By this isolation procedure it has been possible to estimate specifically the concentrations of prothrombin, fibrinogen, thromboplastic cell component and thromboplastic plasma component. This communication contains data demonstrating significant elevation of thromboplastic plasma component in patients with acute pancreatitis and in dogs with experimental pancreatitis. Serum amylase, prothrombin and fibrinogen results are also reported.

Methods. About 8 ml of blood were obtained by venipuncture and allowed to clot. The syringe was replaced by one containing

1.5 ml 4% sodium citrate solution and approximately 15 ml of blood were collected. The citrated blood was centrifuged in calibrated 15 ml graduated tubes at 1°C, 1,650 g, for 30 minutes. Fibrinogen, thromboplastic plasma component and prothrombin were determined on the protein fractions obtained from 4 ml of plasma by a low temperature alcohol procedure(7). Serum amylase was determined by Somogyi's saccharogenic technic(8).

Results. Normal values. Serum amylase levels below 60 and above 200 are considered abnormal in human beings(9). Normal plasma fibrinogen and prothrombin concentrations are respectively 0.26, S.D. $\pm$ 0.05% and 81.8, S.D. $\pm$ 14.4 units/ml(7). In the present study the mean thromboplastic plasma component (TPC) concentration in 31 normal men and women was found to be 60.4, S.D. $\pm$ 34.8 units/ml. In 13 healthy untreated adult dogs weighing between 9 and 18 kg the means and ranges were: amylase, 1309 (695-1970) units/100 ml; TPC, 1112 (64-3848) units/ml; fibrinogen, 0.329% (0.139-0.585%); and prothrombin, 39.3 (24.3-58.8) units/ml. Therefore in such animals the average amy-

* Supported in part by grants from the National Institutes of Health and the Comly Fund.

[†] In partial fulfillment of the requirements for Master of Medical Science degree in Surgery; Dr. Waite, member of Public Health Service, National Institutes of Health.

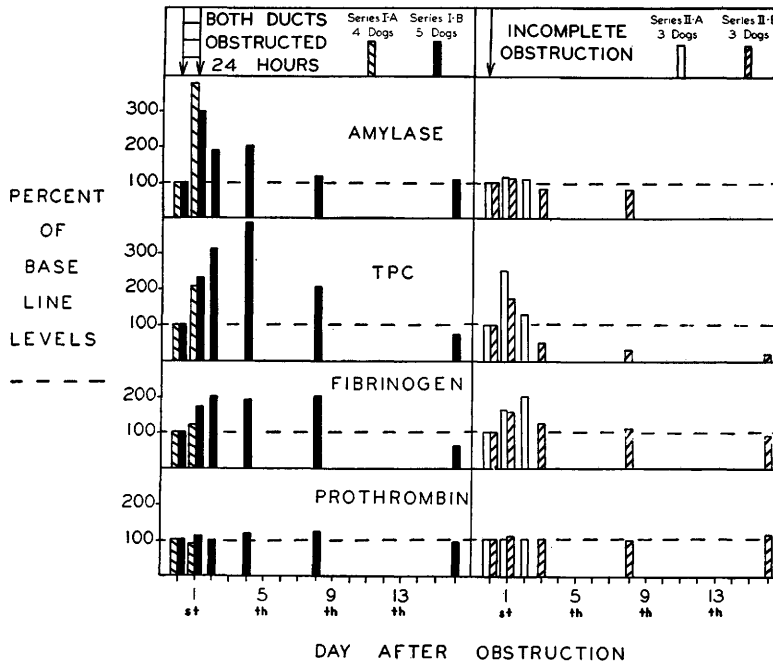


FIG. 1. Changes in serum amylase and plasma coagulation factors in dogs following obstruction of pancreatic ducts.

lase and TPC levels are about 10 and 20 times higher respectively than in human beings. The prothrombin level in dogs is about one-half that in human beings.

Experimental pancreatitis. Sublethal attacks of pancreatitis were produced in dogs by a technic previously described in detail(10). The major and minor pancreatic ducts were each encircled with loops of nylon tubing 1.5 mm in diameter. After a 3 weeks recovery period from the effects of surgery, blood specimens were obtained for baseline values (zero day, Fig. 1). Then, in the dogs of Series I-A and I-B, both ducts were obstructed by tying the ends of the nylon loops under tension. After 24 hours, the nylon loops were released and blood specimens were immediately obtained: The results thus found are shown as 1st day values in the left half of Fig. 1. In all 9 dogs the amylase level was 3.3 (range, 2-5) times greater on the 1st day after 24 hours duct obstruction. The values approached baseline levels by the 8th day. The TPC level in all 9 dogs was 2.2 (range 2-4) times greater on the 1st day. The peak was reached on the 4th day, when the TPC level

was 3.7 (range 2-8) times greater than the baseline, and was still elevated on the 8th day. It was slightly below baseline level on the 16th day. The fibrinogen level increased to a 2-fold level in about the 2nd day after obstruction and remained at this level on the 8th day. The prothrombin level did not change significantly. In the dogs of Series I-A and I-B, which were sacrificed, autopsy showed gross and histologic evidence of pancreatitis, characterized by fat necrosis, parenchymatous degeneration and interstitial inflammatory infiltration.

In the right half of Fig. 1 are the results obtained on 6 dogs (Series II-A and II-B) in which only one nylon loop was tied or in which autopsy disclosed that one or both nylon loops had broken. Under these conditions the serum amylase values did not deviate more than one-fourth of the baseline levels. The TPC and fibrinogen levels changed but not to the degree in respect to extent and duration demonstrated in the dogs whose ducts were completely obstructed for 24 hours. Moreover, the TPC peak was reached on the 1st day and by the 2nd day was almost back

TABLE I. Levels of Serum Amylase and of Plasma Coagulation Factors Associated with 6 Attacks of Acute Interstitial Pancreatitis in 5 Patients.

Case No.	Age, sex	Dates	Serum amylase, Somogyi, units/100 ml	Thromboplastic plasma component: TPC, units/ml	Fibrinogen, %	Prothrombin, units/ml
1283	78 ♂	9/17	744	492	.431	46.7
1347	65 ♂	1/13	498	—	—	—
		1/17	67	1302	.497	62.3
1366	39 ♂	2/25	704	112	.485	81.3
		2/26	304	83	.528	74.3
		2/27	93	137	.596	76.5
		3/2	62	311	.728	78.2
		3/3	77	299	.658	79.1
		3/4	94	279	.692	83.0
1325	70 ♀	12/15	1119	—	—	—
		12/18	406	1321	.615	64.4
1334	57 ♀	1/21	1449	—	—	—
		1/22	183	585	.316	83.8
		1/23	136	178	.288	78.1
		1/26	133	158	.280	80.8
		1/28	1107	382	.177	76.1
		1/28*	667	315	.217	77.9
		1/29	307	565	.452	71.4
		1/30	82	1123	.697	76.9
		2/2	67	549	.626	76.8
		2/5	98	145	.421	89.5
		2/5 *	59	143	.419	80.0
		2/6 †	92	238	.412	78.4

* Specimens collected 4 p.m.; all others between 8 and 9 a.m.

† 24 hr after a cholangiogram.

to the baseline level: The 4th, 8th and 16th day levels were low.

Pancreatitis in patients. The levels of plasma coagulation factors in 5 patients with acute interstitial pancreatitis and elevated serum amylase are presented in Table I. Following all 6 attacks the TPC values were 5 to 22 times the normal mean. The peak in the TPC elevation occurred several days after that of the amylase level. The TPC level remained high for over a week. There was slight to moderate elevation of plasma fibrinogen. All prothrombin results were well within the normal range except for a moderate diminution in Case 1283.

In addition 6 cases were studied on whom the diagnosis of chronic pancreatitis had been made. The investigations and the ranges of results were: amylase, 24-125 units; TPC, 14-77 units; fibrinogen, 0.20-0.57%; and prothrombin, 24-71 units.

Discussion. In a variety of pathologic conditions studied thus far in this laboratory, non-fatal acute pancreatitis is the first in which

consistently marked TPC elevations have been demonstrated. A definite deficiency of this factor in hemophilic blood plasma was previously reported(11). TPC is an essential factor in the blood coagulation mechanism because together with thromboplastic cell component, a lipoprotein of blood cells, it quantitatively converts prothrombin to thrombin(7). In view of the foregoing, an excess of TPC could be interpreted as increasing the coagulability of blood. However, the importance of this finding in respect to thrombosis associated with pancreatic diseases can be determined only by further investigations.

Summary. Following 6 attacks of acute interstitial pancreatitis in 5 patients and the obstruction of both pancreatic ducts in 9 dogs, the thromboplastic plasma component showed a consistently marked elevation. The significance of this observation is discussed. The increase in plasma fibrinogen concentration in acute pancreatitis was confirmed. Prothrombin did not vary significantly. In addition the levels of plasma coagulation fac-

tors in 6 patients with chronic pancreatitis, in 6 dogs with incomplete obstruction of the pancreatic ducts and in 13 normal untreated dogs are reported. The coagulation factors were determined quantitatively on plasma fractions obtained by a low temperature procedure. Amylase determinations were done on all specimens.

The authors are grateful to Mrs. Dolores M. Johnston, Miss Marilyn J. Herritt, Miss Elizabeth P. Lorey and Mrs. Anne W. Tatum for the determination of the coagulation factors; to Miss Rachel B. Earp, Miss Donna F. Bitzel, Miss Margaret A. Delaney and Miss Martha I. Walters for the amylase determinations; and to Dr. Robert M. Zollinger for making his patients available for this study.

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Received April 10, 1953. P.S.E.B.M., 1953, v83.

A Simple Technic for Demonstrating Plaque Formation with Virus of Vaccinia.* (20378)

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A technic for the demonstration of focal necrotic areas (plaques) by animal viruses would provide a more accurate means of titration. It would also be a valuable tool for studying their growth characteristics and genetic properties. In addition, it might be helpful in isolating mutant viruses and in studying antiviral substances. There has been publication on such a technic. Gey and Bang(1) stated briefly that they had been able to produce focal necrosis in static slide cultures of a rat sarcoma with the virus of Eastern equine encephalomyelitis. Recently Dulbecco(2) reported in detail on plaque formation in chick embryo tissue cultures by the viruses of Western equine encephalomyelitis and Newcastle Disease. The present paper describes a simple, readily reproducible technic whereby plaque formation has been

consistently obtained with the virus of vaccinia.

Materials and methods. The cell suspension. Five, 11-day-old chick embryos were decapitated, washed twice with Gey's saline (GS) and pressed into 15 ml of GS through a wire mesh strainer, having 15 strands per cm. This material was centrifuged at $110 \times G$ for 30 seconds and the resulting supernatant was discarded. The sediment was resuspended, pipetted rapidly 20 times with a 10 ml pipette and centrifuged at $110 \times G$ for 15 seconds. The supernatant was then removed and allowed to stand for 20 minutes, during which time the large particles sedimented into the bottom 1 ml. This was discarded and the resulting preparation consisted, for the most part, of a suspension of single cells. The cell concentration of the suspension was then determined in a hemocytometer and an amount added to the nutrient fluid which resulted in a final cell concentration of 8×10^6 cells per ml. This usually required about 10 ml of the cell

* This work was supported by funds from the National Cancer Institute of the U. S. Public Health Service, American Cancer Society and the Damon Runyon Memorial Fund for Cancer Research.