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Disturbances in Blood Coagulation and Fibrinolysis under the Influence of Pancreatic Juice

by

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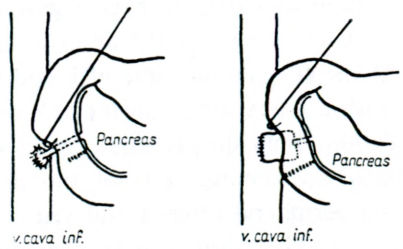
In experiments previously reported [13], in which the toxic effect of the pancreatic juice was studied, an intravenous administration of large amounts of the juice was found to induce death of the animal with symptoms of hemorrhagic diathesis. This finding prompted us to investigate in detail the changes in the coagulation system induced by the pancreatic juice circulating in the blood.

Materials and methods

The experiments were performed with 25 mongrel dogs weighing from 14 to 20 kg. Group I consisted of 10, group II — of 8, and group III — of 7 dogs. In the first group (control) the coagulation system was examined in absolutely healthy dogs to obtain average standards of blood coagulation.

Experiments of group II were aimed at investigation of the coagulation system under a continuous intravenous supply of inactive pancreatic juice, i.e. juice contained in the pancreatic ducts



and not in contact with the duodenal mucosa. The end portion of the pancreatic duct together with the papilla was inserted into the vena cava inferior (Fig. 1); in this way the total amount of juice produced by the pancreas passed directly into the circulation.

To determine disturbances in the coagulation system after administration of active pancreatic juice, in experiments of group III, the pancreatic duct was inserted into the vena cava inferior together with a 2-cm. segment of the duodenum (Fig. 2); in this way, owing to contact with the duodenal mucosa, activated pancreatic juice passed into the circulation.

In animals of all groups the following tests were performed at 2-day intervals:

1. Whole blood clotting time by the Lee White method.
2. Calcium clotting time.
3. Plasma thrombin time. Thrombin was used diluted to a concentration which, with the donor's oxalated plasma, gave coagulation time equal to 15 sec. in the system: 0.2 ml. plasma +0.1 ml. thrombin.
4. Fibrinogen by the Quick method [12].
5. One-stage prothrombin time by the Quick method [11] as expressed by the prothrombin index.
6. Factor VII by the method introduced by Koller et al. [3].
7. Factor V by the Wolf method [16].
8. Whole clot lysis by the method described by Kopeć and Niewiarowski [4].

The result was expressed as percentual loss of fibrin after 24-hour incubation of the clot at room temperature.

9. Euglobulin lysis time by the method developed by Kowarzyk and Buluk [6]. The results were expressed as the euglobulin index $= \frac{1{,}000}{\text{fibrinolysis time in min.}}$
10. Plasminogen by the method of Kowalski, Latałło, and Niewiarowski [5]. The results were expressed as the plasminogen index $= \frac{\text{time of lysis of the clot with the donor's plasma.}}{\text{time of lysis of the clot with the plasma investigated}}$
11. Antiplasmin by the method of Niewiarowska and Węgrzynowicz [8]. The results were expressed as the antiplasmin index $= \frac{\text{time of lysis of the clot with the plasma investigated.}}{\text{time of lysis of the clot with the donor's plasma}}$
12. Immediate antithrombin according to Niewiarowski and Kowalski [9].
13. Erythrocyte count.
14. Platelet count.

Results

The survival time of dogs in group II amounted on the average to 8 days and in group III to 2—4 days, and was probably related to the activity of the pancreatic juice secreted. This was manifested by an increase in the level of pancreatic juice enzymes in blood: diastase up to 16.384 μ (normal level 128 μ), lipase up to 6.0 ml. of 0.05 NaOH (normal level up to 0.5 ml.), and the proteolytic activity up to 46 gamma (normal up to 10 γ). Changes in the coagulation system appeared as early as the first day of observation and then increased gradually. The average values of these changes on the last day of life of the animal are presented in the Table.

In animals of group II the coagulation time of blood and plasma after recalcification was prolonged, with a co-existing tendency to coagulation in large blood vessels with slower circulation. Immediately after death, numerous clots could be found in both heart ventricles, forming casts of the ventricles. Similar changes were found to occur in the peripheral arteries and veins: the aorta, and the superior, and inferior vena cava. The fibrinogen level gradually increased to reach its highest values before death. Prothrombin and proaccelerin deficiency was only slight, while the decrease in proconvertin level was highly significant. A slightly higher fibrinolytic activity was expressed by an 18 per cent loss of fibrin after 24-hour incubation, while fibrinolysis in euglobulins was highly inhibited with a distinct increase in fibrinogen level. The lack of fibrinolysis activation occurred together with higher plasminogen index and a considerable increase in antiplasmin titer.

TABLE

		Group I (Healthy dogs)	Group II	Group III
1	Clotting time	(3'30''/±42'') (3'10''—3'50'')	19'47''(±6'10'') (10'30''—22')	15'30''/±8'30 (7'—32')
2	Calcium clotting time	58''(±8'') (44''—62'')	138''(±20'') (75''—185'')	122''(±40'') (108''—225'')
3	Thrombin time	15''	16.5'' (±1,0'') (15''—17.5'')	no clot small fibers
4	Fibrinogen mg./ml.	5.28 (±1.1) (4.9—6.1)	8.40 (±2.98) (6.0—13.2)	traces
5	Quick prothrombin index	100% (±1.8%)	76% (±3.1%) (72%—79%)	48% (±3.9%) (26%—52%)
6	Factor VII—% of normal level	100% (±1.7%)	45% (±4.0%) (32%—49.5%)	48% (±6.6%) (18%—56%)
7	Factor V—% of normal level	100% (±1.5%)	92% (±1.6) (91%—93%)	26% (±2.2%) (12%—28%)
8	Fibrinolysis in plasma % of fibrin loss	does not occur	18.5% (±2.0%) (12.0%—21%)	100% total
9	Euglobulin fibrinolytic activity Euglobulin index	19.2 (±1.8) (18.2—22.2)	4.1 (±1.1) (3.8—4.9)	66.6 (±3.1) (51.2—76.2)
10	Plasminogen index	1.0 (±0.1) (0.98—1.1)	1.5 (±0.15) (1.45—1.55)	0.06 (±0.04) (0.04—0.1)
11	Antiplasmin index	1.0 (±0.05)	24.0 (±1.6) (21.5—26.0)	1.09 (±0.06) (1.04—1.12)
12	Antithrombin VI	15''	13''(±0.5'') (12''—14'')	31''(±6'') (21''—33'')
13	Erythrocyte count	5.222.000 (±118.000) (5.100.000)— —5.340.000)	4.540.000. (±270.000) (4.280.000— —4.880.000)	6.440.000 (±540.000) (5.870.000 —6.990.000)
14	Platelet count	265.740 (±5.640) (258.000— —273.000)	195.200 (±50.500) (143.640— —204.300)	123.440(±11.560) (111.000— —139.900)

The results were entirely different in group III in which the active pancreatic juice was infused into the blood. In animals of this group open fibrinolytic diathesis, the lack of fibrinogen in the circulating blood, thrombin time infinitely long, and a decrease in prothrombin, factors V and VII and particularly factor V senstive to plasmin action, were observed. Small fibrin fibers precipitated by highly active thrombin from full plasma were, immediately digested, the time of lysis in euglobulins amounting to several minutes. Because of a very low amount of fibrinogen, it is difficult to draw any conclusions as to the true activity in euglobulins. A large decrease in the plasminogen index also was evidence that this proenzyme was consumed in the process of activation of the fibrinolytic system. No significant deviation in antiplasmin activity was observed in this group.

Discussion

As is evident from the experiments just presented, in cases when inactive pancreatic juice was supplied directly into the circulation (group II) blood hypercoagulability continuous increase in fibrinogen, inhibition of fibrinolysis in euglobulins, an increase in the plasminogen index, and high antiplasmin titer could be observed. Such a high amount of antiplasmin seems to originate from the pancreatic juice. The prolonged time of blood clotting and recalcification might be due to passing into the blood of certain inhibitors or to a deficiency of other factors of the first phase of the coagulation system.

In cases when active pancreatic juice passed into the blood (group III) the acute fibrinolytic diathesis was observed which was manifested by a lack of fibrinogen in the blood a decrease in prothrombin, factor V and VII, shorter time of lysis in euglobulins, total fibrinolysis in the full plasma, and a lower plasminogen index.

In the course of fibrinogen digestion by plasmin, products of partial fibrinogen degradation are formed, exhibiting properties of coagulation inhibitors: antithrombin VI and a factor inhibiting the formation of plasma thromboplastin [9], [10]. A higher rate of fibrinolysis could be concluded from the presence of immediate antithrombin in the plasma.

As the results presented indicate, changes in the coagulation system can be induced by enzymes of the pancreatic juice. This observation makes it possible to understand in some measure the disturbances in humans in the case of acute pancreatic necrosis, when large amounts of pancreatic enzymes pass into the blood. The clinical changes are accompanied by certain disturbances in the blood coagulation system clinically manifested by hypercoagulability [14] or, in cases of prolonged necrosis of the pancreas, by a tendency to hemorrhages with a decrease in the level of prothrombin and fibrinogen [1], [15]. The disturbances are ascribed by some authors to the action of trypsin [2], [7].

However, the origin of an excess of antiplasmins, which is of high importance from the standpoint of hematology, remains to be explained by further studies.

Conclusions

1. Blood hypercoagulability and an increase in the level of fibrinogen and antiplasmins are induced by large amounts of inactive pancreatic juice passing directly into the circulation.

2. Fibrinolytic diathesis is induced by activated pancreatic juice passing into the circulation.

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REFERENCES

[1] S. Borgström, *On the prothrombin index in acute affections of the pancreas*, Acta Chir. Scand., **90** (1944), 419.

[2] J. A. Innerfield, A. Schwartz, A. A. Angrist, *Intravenous trypsin: its anticoagulant fibrinolytic and thrombolytic effects*, J. Clin. Invest., **31** (1952), 1049.

[3] F. Koller, A. Loeliger, F. Duckert, *Experiments on new clotting factor (Factor VII)*, Acta Hematol., **5** (1951), 1.

[4] M. Kopeć, S. Niewiarowski, *Czynność fibrynolityczna osocza osób zdrowych* [in Polish], [*Fibrinolytic activity of the plasma of healthy humans*], Pol. Arch. Med. Wewn., **26** (1965), 1321.

[5] E. Kowalski, Z. Latałło, S. Niewiarowski, *Plazminogen i plazmina. I. Badania nad aktywacją plazminogenu i inaktywacją plazminy* [in Polish] *Plasminogen and plasmin I. Studies on plasminogen activation and plasmin inactivation*, Acta Bioch. Polon., **3** (1956), 387.

[6] H. Kowarzyk, K. Buluk, *Postępy badań nad krzepnięciem krwi* [in Polish] *Advances in studies on blood coagulation*. Postępy Hig. i Med. Dośw., **2** (1950), 1.

[7] E. Lasher, M. Mc Cabe, *Change in coagulation time associated with experimental acute pancreatitis in dogs*, Arch. Surg., **60** (1950), 164.

[8] M. Niewiarowska, Z. Węgrzynowicz, *Influence of dicumarol derivatives of the plasma fibrinolytic system*, Thrombosis et Diath. Haemorrh., **3** (1959), 279.

[9] S. Niewiarowski, E. Kowalski, *Un nouvel anticoagulant derivé du fibrinogène*, Revue d'Hematologie, **13** (1958), 320.

[10] S. Niewiarowski, Z. Latałło, J. Stachurska, *Apparition d'un inhibiteur de la thromboplastinoformation au cours de la proteolyse du fibrinogène*, ibid., **14** (1959), 118.

[11] A. J. Quick, *The prothrombine in haemophilia and obstructive jaundice*, J. Biol. Chem., **72** (1935), 109.

[12] — , *The physiology and pathology of hemostasis*, Philadelphia, 1957.

[13] W. Rowiński, A. Michalski, H. Łukasiewicz, W. Olszewski and J. Nielubowicz, *Badania nad patogenezą ostrej martwicy trzustki. I. Toksyczne działanie soku trzustkowego podawanego dożylnie* [in Polish] [*Studies on the pathogenesis of acute pancreatic necrosis. I. Toxic effect of pancreatic juice administered intravenously*], Pol. Przegl. Chirurg., [in press].

[14] G. Y. Shinowara, *Hypercoagulability in acute pancreatitis*, Amer. Journ. of Surg., **105** (1963), 714.

[15] J. Storer, P. Kazdan, *The relation of the pancreas to blood coagulation*, Surgery, **33** (1953), 683.

[16] P. Wolf, *A modification for routine laboratory use Stefanini's method of estimation factor V activity in human oxalated plasma*. J. Clin. Path., **6** (1953), 34.