Lactic acid production (μg/mg dry material/h for spleen, bone marrow, placenta and μg/10^7 cells/h for leucocytes) in the tested tissues of endotoxin- and saline-treated pregnant and non-pregnant rats 24 h after treatment. The numbers of samples are in brackets

Group	Treatment	Spleen		Bone marrow		Leucocyte		Placenta	
		$\overline{X}$	$s_{\overline{x}}$	$\overline{X}$	$s_{\overline{x}}$	$\overline{X}$	$s_{\overline{x}}$	$\overline{X}$	$s_{\overline{x}}$
Non-pregnant (NP.)	Saline (S.)	8.3 (11)	0.50	10.3 (5)	0.64	4.1 (5)	0.3	–	–
	Endotoxin (E.)	10.3 (15)	0.38	16.8 (5)	0.49	5.1 (5)	0.2	–	–
Pregnant (P.)	Saline (S.)	8.6 (8)	0.60	12.5 (5)	0.73	4.7 (5)	0.2	11.6 (20)	0.41
	Endotoxin (E.)	11.4 (10)	0.55	19.7 (5)	0.64	7.1 (5)	0.6	8.7 (14)	0.42
Difference between	NP.S. and NP.E.		$p < 0.01$		$p < 0.001$		$p < 0.05$		
	P.S. and P.E.		$p < 0.01$		$p < 0.001$		$p < 0.01$		$p \ll 0.001$
	NP.E. and P.E.		$p > 0.1$		$p < 0.01$		$p < 0,05$		
	NP.S. and P.S.				$p > 0.05$				

marrow samples were taken from the tibia and femur. Leucocytes and bone marrow cells from 2 animals were pooled. Spleens were studied separately for each individual animal. 3 pooled placentas per animal served as individual samples. Tissue samples were weighed and minced prior to their transfer into an adequate volume of Krebs-Henseleit solution containing 200 mg% glucose. The lactic acid production was measured by the method of DIESCHE and LÁSZLO[12]. Lactic acid production is expressed as μg/mg dry material/h and μg/10^7 cell/h for spleen, bone marrow, placenta and leucocytes, respectively.

Results. Lactic acid production of spleens, bone marrow cells, leucocytes and placentas of endotoxin treated pregnant and non-pregnant rats is shown in the Table.

When tested after 24 h following treatment with endotoxin, the lactic acid production of the placentas and of other tissues (spleen, bone marrow and leucocytes) exhibited a considerable difference. Aerobic glycolysis was inhibited in placentas, while stimulated in all tissues tested.

In endotoxin-treated animals, the rate of lactic acid production was significantly more enhanced in bone marrow cells and leucocytes of pregnant rats than in the non-pregnant ones.

Zusammenfassung. Die Milchsäureproduktion ist im Knochenmark und in den Leukozyten trächtiger Tiere infolge Endotoxineinwirkung signifikant höher als bei Kontrolltieren. Während sich die Milchsäureproduktion endotoxinbehandelter Plazenten infolge Dauerhemmung der aeroben Glykolyse völlig von allen untersuchten Geweben unterscheidet, bleibt der Sauerstoffverbrauch unverändert.

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21st November 1966.

[12] Z. DIESCHE and D. LÁSZLÓ, Biochem. Z. *187*, 344 (1927).

Clotting Factors of the Primary Aqueous Humor of the Rabbit's Eye

According to several authors, primary aqueous humor (PAH) accelerates the clotting time of the whole blood and of the plasma[1,2] and contains various clotting factors, e.g. prothrombin[2], factor V and factor VII[2,3]. PANDOLFI and NILSSON[4] found that PAH contains plasminogen and proactivator but no measurable amounts of other clotting factors. The purpose of the present study was to characterize more precisely the nature of the clot-promoting substances of this fluid.

PAH was drawn from the anterior chamber of the rabbit's eye by means of a puncture with a s.c. needle. About 0.1–0.3 ml of the transparent fluid was obtained from each eye. Samples contaminated with blood were discarded.

The following investigations were performed:

(1) The influence of PAH on the recalcification time of fresh, human, platelet-poor plasma (PPP) was studied in the system: 0.1 ml of PPP + 0.1 ml of PAH (or 0.9% NaCl) + 0.1 ml of 0.025 *M* $CaCl_2$. The recalcification time of 99 control samples amounted on an average to 214 sec (range: 120–360 sec). The recalcification time of 124 samples containing PAH amounted on an average to 128 sec (range: 35–240 sec). All but 18 fluid samples significantly shortened the clotting time of the recalcified

[1] F. RINTELEN and J. OERI, Ber. Versamm. dt. ophthal. Ges. *56*, 305 (1950).
[2] A. VANINI, Rass. ital. Ottal. *26*, 265 (1957).
[3] M. APPELMANS, Bull. Soc. belge Ophtal. 241 (1955).
[4] M. PANDOLFI and I. M. NILSSON, Int. Congr. Haemat. 69 (1964).

human plasma. Subsequent studies revealed that the clot-promoting activity is completely inactivated during storage of the fluid at room temperature for a few h, or at 4 °C for 24 h or at − 10 °C for 170 h. The activity is also removed by shaking PAH with barium sulphate, ether or chloroform. The experiment presented in Table I shows that clot acceleration occurs also where cephalin in an optimal concentration has been added to the system.

(2) The presence of various clotting factors in PAH was tested using routine one stage methods. Deficient plasmas were received from Prof. P. A. Owren of the Institute for Thrombosis Research (deficient in factors V and VII), from Dr. E. A. Loeliger of the Akademisch Ziekenhuis, Leiden (deficient in factor XII), and from one of the authors (S.N.) from children treated in Warsaw University Children's Clinic (deficient in factors VIII, IX and X). The deficient plasmas were also prepared artificially: factor XI and XII deficient by the method of Waaler[5], factor V deficient by the method of Wolf[6], factor II deficient by the method of Soulier and Larrieu[7]. All samples were stored as freeze dried or in deep freeze.

It has been found that PAH shows a high activity of factors VIII, IX, XI and XII. These activities were considerably higher than those of human blood plasma diluted 1:10. Similarly factor V activity of PAH was high when tested in the intrinsic system (with cephalin but without tissue thromboplastin). The activity of prothrombin (tested by the method of Soulier and Larrieu) in PAH was about 10 times lower than in human plasma. The activity of factor V and VII tested in the extrinsic system were several times lower than in human plasma. The factor X activity, as tested in the extrinsic system, was absent. However, PAH shortened the recalcification time of factor X deficient plasma. PAH did not contain fibronogen. The samples did not clot even after addition of a large amount of thrombin. The fresh fluid contains low concentrations of thrombin (lower than 0.1 u/ml). 0.1 ml of fluid clotted 0.1 ml of 0.2% purified fibrinogen in about 30 min.

(3) The influence of PAH on the intrinsic clotting has been tested using the thromboplastin generation test according to Duckert et al.[8]. The system generating 'thromboplastin' was composed of platelet extract (or cephalin), $BaSO_4$ adsorbed plasma, old serum, and calcium chloride. It has been found that PAH accelerates formation of 'thromboplastin' if added to the whole system. It can be seen from Table II that PAH restores the activity of the system deprived of old serum and it replaces also, in part, the activities of $BaSO_4$ adsorbed plasma, platelet extract or serum. These experiments show that the activity of PAH in the intrinsic blood-clotting system is not specific.

It is possible that the intraocular fluid contains high concentration of various clotting factors, particularly factors VIII, IX, and XII which may be selectively filtered from blood or secreted. Another explanation is that PAH contains an activator of 'intrinsic clotting' which may be different from other plasma and tissue clotting factors. This activator may act directly on prothrombin or on factor X. PAH clotting activator resembles in some aspects urinary procoagulant described by von Kaulla[9]. The latter can substitute for factors VIII, IX, XI and XII but not for factors I, II, V, VII, or X[10].

The clotting activator of the PAH may play a significant role in the local hemostasis of the eye. It may also be responsible for the formation of fibrin deposits in the course of various eye diseases.

Table I. Influence of PAH on the recalcification time of platelet-poor plasma (PPP)

Solution added to PPP	Clotting time in sec
0.9% NaCl	306
0.9% NaCl + cephalin	85
PAH + 0.9% NaCl	168
PAH + cephalin	53

Table II. Activity of PAH as tested in thromboplastin generation test

Component to be omitted or replaced	Activity of the 'thromboplastin' generated after 7 min (clotting time, sec)		
	Whole system	One component omitted	One component replaced by PAH
Platelet extract	20	54	42
$BaSO_4$ adsorbed plasma	20	44	26
Old serum	20	33	23
Cephalin[a]	15	120	29

[a] In this experiment cephalin prepared from rabbit brain has been used instead of platelet extracts. Other preparations of $BaSO_4$ adsorbed plasma and of old serum were also used.

Résumé. On a constaté que l'humeur aqueuse primaire (PAH) de l'œil du lapin racourcit le temps de coagulation du plasma humain. Il contient une proportion élevée de facteurs de coagulation, en particulier les facteurs VIII, IX, XI et XII. Il est possible que la propriété coagulante du PAH est due à la présence d'un activateur spécifique agissant directement sur la prothrombine ou sur le facteur X.

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Department of Ophtalmology and Department of Physiological Chemistry, Medical School, Bialystok (Poland), 15th December 1966.

[5] B. Waaler, Scand. J. clin. Lab. Invest. Suppl. *11*, 37 (1959).
[6] P. Wolf, J. clin. Path. *6*, 34 (1959).
[7] J. P. Soulier and M. J. Larrieu, Sang *23*, 549 (1952).
[8] F. Duckert, P. Fluckinger, H. Isenschmid, M. Matter, J. Vogel-Meng and F. Koller, Acta haemat. *12*, 197 (1954).
[9] K. N. von Kaulla, Proc. Soc. exp. Biol. Med. *91*, 543 (1956).
[10] J. H. Lewis, in *The Hemophilias, International Symposium* (Ed. K. M. Brinkhous; University Press of North Carolina, Chapel Hill, N.C. 1964), p. 185.