13531 P

Loss of Prothrombin Activity in Plasma Exposed to Air Currents.

L. M. TOCANTINS.

From the Division of Hematology, Department of Medicine, Jefferson Medical College, Philadelphia, Pa.

During the early summer of 1941 it was noticed that oxalated plasma collected for the quantitative determination of prothrombin by the Quick method[1] underwent a marked decrease in activity after standing a few hours, uncovered, directly in the cool draft of a York air-conditioning unit. The experiments reported indicate that this decrease in prothrombin activity is due to rapid evaporation and loss of CO_2 by the plasma. Addition of CO_2 even by the simple method of saturating the plasma with expired air restores prothrombin activity to its initial level.

1. Changes in prothrombin time of plasma exposed at 38°C for 1 hour to a continuous draft from an air conditioning machine at a distance of 8 feet.

Another specimen of plasma was placed in a 15 cc centrifuge tube, uncorked, 30 cm in front of a fan. The prothrombin time before exposure was 16.8 sec. After 2 hours of exposure it was 53.7 sec. A 1-1 dilution of this plasma with .85% sodium chloride gave a prothrombin time of 130.9 sec. Exposure of plasmas at room temperature to still air for 4 hours results in only a slight decrease in prothrombin activity. Analogous results have been obtained with oxalated or citrated rabbit and dog plasma.

2. Effect of exposure to oxygen or CO_2 on the prothrombin activity of fresh plasma. Plasma was separated under oil from oxalated dog blood, placed in a 15 cc centrifuge tube and oxygen

TABLE I.

July 20, 1941. Plasmas separated from human oxalated blood (0.1 vol. of 1.34% sodium oxalate solution added to the blood). 0.1 cc plasma, 0.1 cc rabbit brain thromboplastin, 0.1 cc 0.025 M $CaCl_2$.

Specimen No.	Prothrombin time (sec) at		
	4.45 p.m.	5.20 p.m.	6.00 p.m.
1	17.6	20.4	21.8
2	19.9	22.4	
3	20.3	22.2	24.2

[1] Quick, A. J., Stanley-Brown, M., and Bancroft, F. W., *Am. J. Med. Sci.*, 1935, **190**, 501.

bubbled through the plasma for 1 minute. No significant change took place in the prothrombin time under these conditions, in agreement with the findings of Andrus, Lord and Kauer.[2] Exposure of fresh dog plasma, centrifuged under oil, to CO_2 gas for 1 minute likewise does not produce a significant change in prothrombin activity. Dilution of the plasma with an equal portion of physiological salt solution increases its prothrombin time, which is not altered by subsequent exposure of this diluted plasma to CO_2 gas for 1 minute.

3. Effect of oxygen or CO_2 on the prothrombin activity of plasma previously exposed to an air current.

In carrying out the tests above described one must guard against blowing any of the reagents into the tubes with one's breath and thus inadvertently increase the CO_2 content of the plasma being tested.

Though the H ion concentration of the plasma, when outside of the limits of 7-8, definitely interferes with prothrombin activity, the restoration of activity by CO_2 to partially evaporated plasmas does not seem to depend entirely on a displacement of pH to the normal range.

Andrus, Lord and Kauer[2] found that in blood leaving the lungs there was about 10% less prothrombin than in blood entering them, and concluded that prothrombin was destroyed in the lungs. Loss of CO_2 in the aerated blood probably accounts for the difference observed

TABLE II.

August 4, 1941. Time of clotting of a mixture of 0.1 cc of plasma, 0.1 cc rabbit brain thromboplastin and 0.1 cc 0.025 M $CaCl_2$. O or CO_2 driven by pressure over surface of the plasma, in a closed vessel.

Type of plasma	Prothrombin time (sec)			
	Before evapora.	After evapora.	Oxygen for 1 min	CO_2 for 1 min
Rabbit, Citrated* (.3%)	9.3 (pH 7.9)	12.3 (pH 8.9)	12.5 (pH 8.9)	9.6 (pH 7.4)
Human, Citrated† (.3%)	17.4	21.3		17.3
Dilut. 1-1 with phys. s.s.	(pH 7.6)	(pH 8.2)		(pH 8.0)
Hemophilic, Citrated‡ (.3%)	14.7 (pH 7.7)	75 (pH 9)	70 (pH 9)	15.3 (pH 7.4)
Dog, Oxalated§ (.13%)	9.7	74		

*Stream of moist, CO_2-free air run over the plasma for 1 hr (Loss in plasma volume by evaporation: 8%).

†Stream of dry air run over plasma in tube, 13 mm in diameter, for 2 hours. (Loss in volume by evaporation: 18%.)

‡Stream of dry air run over plasma in a wide bottom flask for 35 min. (Loss in volume by evaporation: 65%.)

§Exposed in a shallow vessel to a fan at 20 cm distance for 3 hours. (Loss in volume by evaporation: 65%.)

[2] Andrus, W. DeW., Lord, J. W., and Kauer, J. T., *Science*, 1940, **91**, 48.

between the two samples. It may be anticipated, furthermore, that asphyxia or hyperventilation (as by forced breathing) will lead to significant fluctuations in the prothrombin activity of the blood. Loss of CO_2 may also account in part for the diminution in prothrombin activity of plasma after being heated to 56°C for 15 minutes, or after standing at 5°C for several days.

Summary. Exposure of citrated or oxalated plasma to an air current is followed by a rapid diminution in its prothrombin activity; this is corrected by the addition of CO_2 and not affected by oxygen.

13532

Hormone-Induced Ovulation in Domestic Fowl.

R. M. FRAPS AND G. M. RILEY.

From the Bureau of Animal Industry, Department of Agriculture, Beltsville Research Center, Beltsville, Md.

Multiple ovulations in hens have been effected in the laboratories of the Bureau of Animal Industry by intravenous administration of a luteinizing preparation after pretreatment with pregnant mare's serum. So far as the authors are aware, this is the first demonstration of direct experimentally induced ovulation in birds.

Ovulation has been induced in a number of mammalian species by administration of anterior pituitary hormones or anterior pituitary-like hormones.[1] Kirschbaum, *et al.*,[2] and Witschi[3] have described full ovarian function in small caged passerine birds following prolonged stimulation with pregnant mare's serum. Increase in rate of ovulation is implied in the results of Clark,[4] Gutowska[5] and Dubowik,[6] but Bates, Lahr and Riddle[7] clearly demonstrated that doses of follicle-stimulating hormone or pregnant mare's serum sufficient to cause marked follicular growth in the laying hen and pigeon also caused cessation of ovulation, usually by the fourth day. These results of Bates, *et al.*,[7] have been confirmed repeatedly in this labora-

1 Hartman, Carl G., Chapter IX, pp. 630-719, in *Sex and Internal Secretions*, Second Edition, Williams & Wilkins, Baltimore, 1939.

2 Kirschbaum, A., Pfeiffer, C. A., Van Heuverswyn, J., and Gardiner, W. U., *Anat. Rec.*, 1939, **75**, 249.

3 Witschi, Emil, *Wilson Bulletin*, 1935, **47**, 177.

4 Clark, L. N., *J. Biol. Chem.*, 1915, **22**, 485.

5 Gutowska, M. S., *Quart. J. Exp. Physiol.*, 1931, **21**, 197.

6 Dubowik, I. A., *Arch. f. Exp. Path. u. Pharmakol.*, 1930, **158**, 154.

7 Bates, R. W., Lahr, E. L., and Riddle, O., *Am. J. Physiol.*, 1935, **111**, 361.