dine N, therefore, is not probable. Since the reagent gives a color reaction with phenylhydrazine, we are inclined to believe that the free NH_2 group of the hydrazine is essential. If there be tuberculostatic metabolites of INH present they either do not react with the reagent, or their amount is too insignificant to influence the readings noticeably. Of importance is, however, that the fluoro compound does not react with p-aminosalicylic acid, which agent is so frequently given together with INH.

The method can easily be adapted to determination of INH in urine. Since the INH concentration in urines is rather high, the urine must be diluted which usually goes to a point, when the yellow color does not interfere. At lower concentrations, the patient's urine may be matched in color with a normal urine at 500 mμ by proper dilution, then the estimation may be carried out in alcoholic or aqueous medium. In the latter case 0.5 cc 0.1N NaOH should be used as alkali. The advantages of the chemical method consist mainly in saving of time. About 8 determinations can be carried out in one hour, so that the results are available on the day when the serum has been taken, whereas the microbiological test requires several weeks.

Summary. A chemical determination of INH in patient's blood has been devised which can be carried out within one hour. The serum is deproteinized with 4 volumes of alcohol, centrifuged, and quantities corresponding to one cc serum are heated with 0.5 cc 0.4% 1-fluoro-2.4-dinitrobenzene solution in alcohol and with 0.1 g borax for 5 minutes at 80°. The developed color is read at a wave length of 500 mμ. The method gives good agreement with results obtained by the microbiological methods within the errors allowed for clinical purposes.

1. Kelly, J. M., Poet, R. B., *Am. Rev. Tuberc.*, 1952, v65, 484.

2. Ballard, C. W., Scott, P. G. W., *Chem. and Indust.*, 1952, v29, 715.

3. Katsu Sestti Yamagushi. *Fol. Pharmacol. Japan*, 1955, v51, 631; Breviaria 37.

4. Monograph: How much INH reaches the tubercle bacilli in your patient? Nat. Jewish Hosp., Denver.

5. Bell, J. Carroll, Riemensnider, D. K., *Am. Rev. Tuberc.*, 1957, v75, 922.

Received March 17, 1958. P.S.E.B.M., 1958, v98.

Plasminogen Activator in Animal Meninges.* (24049)

PETER MOLTKE (Introduced by Tage Astrup)
Biological Institute, Carlsberg Foundation, Copenhagen, Denmark

Presence of an activator of plasminogen in animal tissues was demonstrated by Astrup and Permin(1), and a method for its quantitative estimation in various organs has been published(2). With this method animal and human organs could be divided into groups of high, medium and low concentrations of plasminogen activator, although large individual variations were encountered(3,4). Lewis and Ferguson(5) found moderate fibrinolytic activity in dog meninges caused by an activator of plasminogen. Their method was not quantitative. Using the quantitative method, Roberts and Astrup(6) found large concentrations in meninges of monkeys. We have recently observed surprisingly high fibrinolytic activities of membranes of the brain(7). This observation has prompted a more thorough examination of the meninges.

Methods. Brains from freshly slaughtered, healthy animals were immediately examined or stored at −20° for later use. Dog brains were obtained from dogs used for experiments on vascular surgery (courtesy of Dr. H. H. Wandall). The Rhesus monkey brains were kindly supplied by Dr. Preben von Magnus

* This investigation was supported by grants to Dr. Tage Astrup, Copenhagen, from Josiah Macy, Jr. Fn., N. Y., and National Danish Assn. against Rheumatic Diseases.

TABLE I. Concentration of Plasminogen Activator in Individual Samples of Animal Meninges. Figures express units of activator/g fresh tissue. Obtained from dilution curves where each estimation is an avg of 3 determinations, and calculated by interpolation on a standard curve. Stand. dev., 7%(2). No. of animals investigated in parentheses.

Pig (11)	Ox (8)	Rabbit (4x5)	Dog (4)	Horse (3)				Monkey (4)		
				Pia						
Pia				Front lobe	Occ. lobe	Cerebellum	Chor. plex.	Pia	Dura	Sinus long. sup.
95, 110, 190	200, 300	75	810	1740	2080	1670	550	950	220	600
355, 740, 935	400, 400	150	1230	2000	3300	1320	650	1500	250	429
935, 1110, 1510	450, 470	165	2460	3020	3080		410	2250	325	420
1930, 4255	490, 540	190	4440					1235	140	500
									350 (spinal)	
									700 (cranial)	

of the Polio Department of State Serum Institute. The pia was carefully removed and freed from adhering brain tissue. No attempt was made to remove the vessels. In some animals the dura mater was similarly examined. The tissues were finely cut, and concentration of plasminogen activator estimated as described by Astrup and Albrechtsen(2). In some experiments the tissues were soaked in water for ½ hour in order to remove excess blood, and adhering water was then removed by pressing between filter paper before cutting. No significant differences between results of the 2 procedures were noticed. Activator concentration is expressed in units of the standard preparation used in previous communications from this laboratory(2,3,4,6). In rabbits the material was collected from 5 animals for each estimation which therefore represents an average value.

Results. Variations in concentration (Table I) from species to species and between individuals are in accordance with estimations in other tissues(3,4,6). Some samples from pig, dog and horse contain the highest concentrations of plasminogen activator yet recorded in any tissue. So far concentrations higher than 500 units per gram fresh tissue have only been recorded in samples from a few human organs (uterus, adrenals, lymph nodes, prostate(4)). The differences in concentrations observed in various parts of the pia could be caused by variations in the number of blood vessels. This suggestion is not necessarily contradicted by the lower activity in pia from the chorioid plexus, since the vessels here are to a great extent capillaries. This result is apparently in accordance with the observation by Astrup and Claassen(8) that fibrinolytic activity of the adventitia (of the human aorta) is high with low activity in the tunica media and no activity in the intima. Though smaller arteries have not yet been investigated it seems permissible to assume that endothelium is the part of the vessel showing least fibrinolytic activity. If this explanation also applies to the differences observed between pia, dura and the superior longitudinal sinus is unknown.

Extremely high fibrinolytic activity was also observed in human meninges. Details will be published elsewhere and their possible relation to the pathogenesis of cerebral hemorrhage discussed.

Summary. 1. Concentrations of plasminogen activator in the pia mater from pigs, horses, cows, monkeys, dogs, and rabbits were generally higher than recorded for any other organs in these animals. 2. Different pia sections of the same animal (horse) showed different activator concentrations. 3. Dura mater (monkey) had lower activity than pia. The wall of the superior longitudinal sinus had higher activity than the dura adjacent to the skull.

1. Astrup, T., Permin, P. M., *Nature*, 1947, v159, 681.

2. Astrup, T., Albrechtsen, O. K., *Scand. J. Clin. Lab. Invest.*, 1957, v9, 233.

3. Albrechtsen, O. K., *Acta Physiol. Scand.*, 1957, v39, 284.

4. ———, *Brit. J. Hæmatol.*, 1957, v3, 284.

5. Lewis, J. H., Ferguson, J. H., *J. Clin. Invest.*, 1950, v29, 1059.

6. Roberts, H. H., Astrup, T., *Thromb. Diath. Hæm.*, 1957, v1, 376.

7. Moltke, P., *VI^e Congrès de la Soc. Européenne d'Hématol.*, Copenhagen, 1957, Abst. p104.

8. Astrup, T., Claassen, M., *ibid.*, p101.

Received March 25, 1958. P.S.E.B.M., 1958, v98.

Macroscopic Studies of Platelet Agglutination; Nature of Thrombocyte Agglutinating Activity of Plasma.* (24050)

K. M. BRINKHOUS, E. C. LEROY,† W. P. CORNELL, R. C. BROWN,‡
J. L. HAZLEHURST, AND G. P. VENNART
Department of Pathology, University of North Carolina, Chapel Hill, N. C.

The spontaneous agglutination of platelets is poorly understood. From the earliest work(1) to the present, studies have been based primarily on microscopic observations. This report describes a test by which time and degree of agglutination are determined macroscopically. Optimum conditions for rapid agglutination of platelets are outlined.

Materials and methods. Preparation of sodium citrate, sodium oxalate, citrate-saline and saturated ammonium sulfate solutions has been described(2). Solutions of $CaCl_2$, $MgCl_2$, and $MnCl_2$ (Baker A.R.) were standardized by chloride analysis and diluted to 0.108 M. Buffered isotonic *EDTA* solution was prepared from the di- and trisodium salts of ethylenediamine tetraacetic acid (Geigy). Na_2EDTA and Na_3EDTA concentrations were 0.04086 M and 0.04734 M respectively. pH was 7.38. The solution was isosmotic with 0.156 M NaCl as determined by freezing point depression. Plasmas and platelet preparations were made from normal or hemophilic canine blood. Methods for preparing citrate plasma, oxalate plasma, fibrinogen(2), and plasmas adsorbed with $BaSO_4$ or fuller's earth (f.e.)(3) have been described. For preparation of *resin plasma*, 7 parts of blood were drawn into a syringe containing 1 part moist resin (Na cycle Dowex 50 cation exchange resin, 20-50 mesh). The blood was then passed twice through a column containing 2½ parts resin, at a flow rate of 6 ml/min. After centrifugation (2500 *g*, 20 min), the plasma was passed through a fresh resin column at the same rate. The albumin and globulin fractions were prepared from oxalate plasma using ammonium sulfate and were dialyzed with Visking casing at 4°C until free of sulfate. *Prothrombin* was a citrate eluate of $BaSO_4$ adsorbate of oxalate plasma, dialyzed against citrate-saline for 18 hrs, 4°C, diluted with saline to 50 Iowa units/ml. *Thrombin* was prepared by a modified citrate activation procedure(4). *AHF* was prepared from f.e.-adsorbed plasma by a modified phosphate-citrate technic(5). The test for fibrinogen has been described(3). *Standard platelet suspension*: Silicone-treated glassware was used. Blood, drawn into an equal volume of EDTA solution previously diluted 1:10 with saline (0.9% NaCl), was centrifuged at 4°C 8-10 min, 425 *g*, and the platelet-rich supernate recentrifuged for 5 min, 2075 *g*. The supernatant plasma (EDTA plasma) was decanted. The platelet pellet was suspended by gentle agitation at 20°C in EDTA solution which had been diluted 1:100 with saline. After centrifugation, 2 resuspensions or "washings" were made in citrate-saline. The final suspension, after centrifugation to remove rbc's (7 min, 90 *g*), was counted with the phase microscope and diluted to the desired concentration, usually 4 x 10^5 platelets/cmm. *Citrate platelet suspension*: The standard procedure was modified by using citrate and citrate-saline instead of the 2 dilute EDTA solutions. *Resin platelet suspension*: The initial plasma-resin column (v.s.) was flushed with 50 ml saline. The platelet-rich wash was

* This investigation was supported in part by grant from Nat. Heart Inst., N.I.H., P.H.S.

† Post-Sophomore Research Fellow, N.I.H., P.H.S.

‡ Student Fellow, Natl. Fn. for Infantile Paralysis.