be noted that these findings reflect not only an adrenal defect but also an alteration in renal function. In view of the above observations on hepatic and renal function in the tumor host, it might be expected that decided changes in the dehydrogenase activity of these tissues would be found. While our data indicate a tendency for the dehydrogenase activity of the kidney slices to fall and the liver slices to rise in the tumor group as compared with the controls, these changes are of limited magnitude and the overlap of the absolute values in the 2 groups is considerable.

The present findings are of interest from several aspects: (a) In view of the importance of the homeostatic functions of the liver and kidney(7) and clinical observations linking kidney and liver function; *i.e.*, the hepatorenal syndrome, it is significant to find evidence of a relatively fixed relationship between the dehydrogenase activity of the liver and kidney. (b) The fall in the K/L ratio in the tumor mice of the CFW and A strains is indicative of a systemic alteration in the tumor host. Since this change was found in the mice with small as well as large tumors, it more probably represents an effect of the tumor rather than an expression of cachexia or inanition. (c) The findings are of interest in regard to the incidence of spontaneous tumors within the strains. The most marked differences in the K/L ratios between the control and tumor mice were found in the CFW and A strains. These strains are known to have a very low incidence of spontaneous breast tumors in the virgin state. In contrast, the K/L ratios in the control and tumor mice of the C3H strains were almost identical. This strain is characterized by a very high (90%) incidence of spontaneous tumors in the virgin mice. The dba strain has an intermediate incidence of breast tumors in the virgin females (11-50%). In this strain the difference in the K/L ratios between the control and tumor animals was found to be just below the level of statistical significance.

Summary. The *in vitro* dehydrogenase activity of liver and kidney slices from female control and tumor mice of the CFW, C3H, dba, and A strains was determined and the relative intensity of the kidney to liver values (K/L ratio) was calculated. The K/L ratio was found to be maintained within a relatively narrow range in the different control groups. In the CFW and A mice this value was shifted to a lower value in the presence of a tumor. Such a change occurred to a lesser degree in the dba mice and not at all in the C3H mice.

1. Black, M. M. and Kleiner, I. S., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 437.

2. Zweifach, B. W., Black, M. M., and Shorr, E., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 848.

3. Huggins, C. E., *Cancer Research,* 1949, v9, 321.

4. Greenstein, J. P., *Biochemistry of Cancer,* N. Y., Academic Press, 1947, 316-366.

5. Black, M. M., and Speer, F. D., *Am. J. Clin. Path.,* 1950, v20, 446.

6. Reifenstein, E. C., Jr., Duffy, B. J., and Grossman, M. D., *Gastroenterol.,* 1949, v13, 493.

7. Shorr, E., Zweifach, B. W., and Black, M. M., *Trans. Assn. Am. Physicians,* 1951, v63, 445.

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A Plasminogen Activator in Spontaneously Active Human Blood.*† (20097)

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In the living organism fibrinolytic activity of blood occurs in a number of physiological and pathological conditions(1,2). The high activity found after certain modes of death (3) has recently been studied(4). The specific fibrinolytic effect observed in blood is caused by a strong adsorption of the fibrinolytic substances to fibrin(5). The experi-

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ments also revealed the presence of a plasminogen activator in the active samples.

Methods and materials. Fibrinolytic activity was measured by: 1) *The standard fibrin plate method*(6) in which the substrate contains a large amount of plasminogen. 2) *The heated fibrin plate method*(7) where the plasminogen in the substrate was destroyed by heating the fibrin plates at 85° for 45 minutes.

Fibrinolytically active globulin was obtained from active samples of human plasma by precipitation at pH 5.3 and low ionic strength(8) and redissolved in buffer to the original volume. *Normal human globulin* was prepared from serum in the same way, but dissolved to ½ of the original volume. *Human plasminogen* was precipitated from serum in the same way, redissolved and reprecipitated by dialysis against distilled water for 2 hours (mechanical stirring) and finally dissolved in buffer to 1/10 of the original volume. *Bovine plasminogen.* Freshly prepared bovine fibrinogen(6) was heated at 56° for 5 minutes in a waterbath. After cooling and centrifugation the supernatant was dialyzed against saturated ammonium sulphate, in the cold, overnight. The precipitate was redissolved, dialyzed against buffer and lyophilized. *Buffer.* Sodium diethylbarbiturate buffer (Michaelis) pH 7.75, containing sodium chloride to an ionic strength of 0.15. *Streptokinase.* A sample was kindly placed at our disposal by Dr. Frank Fletcher, Cheshire, England.

Results. Blood from cases of sudden death. Active blood samples from cases of sudden anoxaemic death(4) contain no fibrinogen or fibrin. The activity of the globulins from 13 different cases was compared by means of the two methods. High activities were found on standard fibrin plates (containing plasminogen), while very low activities were found on heated fibrin plates (containing no plasminogen). Addition of plasminogen to the solution of active globulin produced a large increase in activity on heated fibrin plates. On standard fibrin plates a smaller increase was obtained (Fig. 1). These experiments show that the active samples contain a plasminogen activator. Various proteases produce almost identical effects on the standard fibrin plate and on the heated fibrin plate(7). Therefore the effect obtained on standard fibrin plates is caused primarily by activation of the plasminogen contained in the substrate. An estimation of the content of activator in active globulin was obtained on heated fibrin plates as follows: An excess of bovine (A.1) and human (A.2) plasminogen was added to a solution of active globulin and the resulting activities were compared with the enzyme activity (plasmin) of the active globulin (B) and with the activity, which could be produced by maximal activation of normal human globulin by addition of active globulin (C.1) or of streptokinase (C.2) (Table I). The results show that the addition of bovine plasminogen (A.1) produced an amount of plasmin which was about 40 times larger than the amount contained in the original sample of active globulin (B) and about 20 times larger than the maximal amount which could be produced by activation of the sample of normal human globulin. Identical activities were obtained by activation of human globulin with active globulin (C.1) or with streptokinase (C.2). The relatively lower activities obtained by addition of human instead of bovine plasminogen to the active globulin was caused by contamination of the preparation with inhibitory substances.

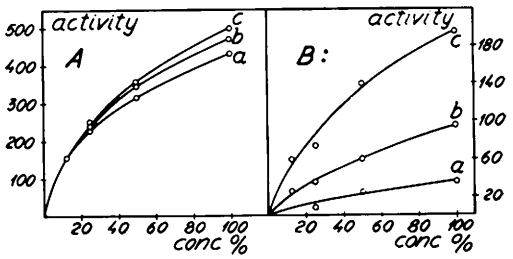

FIG. 1. Dilution curves of mixtures of: a) active globulin and buffer, b) active globulin and human plasminogen and c) active globulin and bovine plasminogen. *A:* Standard fibrin plates (containing plasminogen) *B:* Heated fibrin plates (without plasminogen). *Abscissa:* Relative concentrations of mixtures in %. *Ordinate:* Activity as product of 2 diameters of the lysed zones (mm^2).

Blood from living persons. Samples of active globulin were also obtained from living persons under strain (electrically induced convulsions in psychiatric therapy). Previ-

TABLE I. Comparison between: (A) The Activity Produced by Addition of Plasminogen to Active Globulin; (B) the Activity Produced by Active Globulin Alone; and (C) the Activity Produced by Maximal Activation of Normal Human Globulin with Active Globulin (C. 1) and with Streptokinase (C. 2).

	Mixtures	ml	Measured activities of dilutions 1	½	¼	⅛	1/16	Activities as % of max. activity Uncorrected	Corrected
A. 1	Active globulin	.50							
	Bovine plasminogen	1	196	146	97	64	49	100	400
	Buffer	.50							
A. 2	Active globulin	.50							
	Human plasminogen	1	144	78	56	44	—	52	208
	Buffer	.50							
B.	Active globulin alone		65	49	25	19	—	11	11
C. 1	Human globulin	1							
	Active globulin	.50	94	64	40	32	—	20	20
	Buffer	.50							
C. 2	Human globulin	1							
	Streptokinase	1	81	64	49	36	—	20	20

Substrate: Heated fibrin plates. *Measured activities:* Products of 2 diameters of the lyzed zones (mean of 3 determinations) in mm². *Activities calculated as % of max. activity:* Uncorrected values are obtained by interpolation on curves drawn from measured activities (compare Fig. 1). These percentages are corrected for dilution in order to compare the activities which correspond to plasma concentrations of active globulin and of human globulin. For concentrations of active globulin (A. 1, A. 2, B) and human globulin (C. 1, C. 2) see: Methods and materials. The concentrations of bovine plasminogen (in A. 1), human plasminogen (in A. 2), active globulin (in C. 1) and streptokinase (in C. 2) necessary to yield maximal activities were estimated in separate experiments.

ous experiments had shown that the fibrinolytic substances in blood were strongly adsorbed to fibrin(5). Thus globulins prepared from serum obtained from active blood were completely inactive, while globulins prepared from the oxalated plasma were fibrinolytically active. Even the slightest clotting in the globulin solutions gave erroneous results, when the remaining solution was used for the estimation of fibrinolytic activity. The high lability of fibrinogen in the plasma globulin solutions greatly increased this difficulty and made the fibrin plate method a very suitable method. The amount of activator in plasma globulins from living persons under strain in 10 different cases was determined as described above. A 2-3 times increase in the amount of plasmin could generally be produced by addition of bovine plasminogen to active globulin solutions.

The activator-plasminogen reaction. In some experiments the activities of mixtures of plasminogen with active globulin (from cases of sudden death) were also measured by means of lysis time determinations on bovine fibrin, performed as described in (9). Solutions of active globulin containing appropriate concentrations of human a) and bovine b) plasminogen were left for 15 min. at 37°C. Fibrinogen was added and clotted with thrombin and the lysis times were determined (Fig. 2). Increasing amounts of plasmin were formed by addition of increasing amounts of plasminogen to the active globulin. Similar results were obtained by means of the heated fibrin plate method.

These experiments indicated that the activation was probably a stoicheiometrical reaction. However, the bovine fibrin used is not a suitable substrate to settle this question because of its high plasminogen content. Furthermore the experiments were complicated by the instability of the activator and by a significant content of inhibitory substances in the plasminogen and activator preparations.

Discussion. It is generally accepted that plasmin is produced in blood by activation of a precursor, plasminogen(1). In the human organism fibrinolytic activity is known to develop under various physiological and pathological conditions(1,2), but no conclusive evidence has been presented concerning the

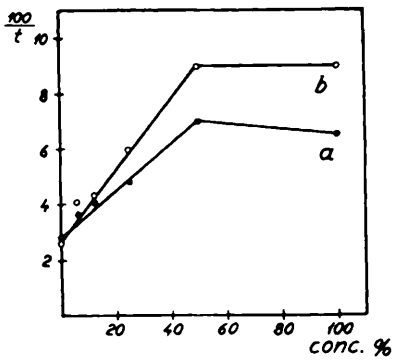

FIG. 2. Lysis times of clotted mixtures of: Active globulin (1.0 ml); thrombin solution (1.0 ml); plasminogen (1.0 ml), and bovine fibrinogen (.05%; 1.0 ml). The mixtures were incubated at 37°C for 15 min. before addition of fibrinogen. *a:* Human and *b:* bovine plasminogen added in increasing concentrations. *Abscissa:* plasminogen concentrations in % of stock solution. *Ordinate:* reciprocal lysis times.

mechanism involved. *Astrup and Permin*(10) demonstrated a physiological tissue activator, fibrinokinase, which later was found to activate plasminogen by a stoicheiometrical reaction(11). An interaction between this factor and the plasminogen in blood has been suggested as a possible explanation(10,1,3,4). Some authors have suggested the presence of an activating principle in blood. *Schmitz* (12) obtained two fractions from dried horse fibrin. Each was slightly active, while an increase in activity was produced after mixing. *Lewis and Ferguson*(13) prepared two fractions from normal dog blood serum. Each was inactive, while increasing activity developed after a period of several weeks, when they were incubated together at 5°C.

In the present work a plasminogen activator is demonstrated in spontaneously active human blood. The amounts present far exceed those necessary for the complete conversion of the plasminogen contained in blood. The activator is soluble and appears rather labile. A similar soluble and labile activator was recently isolated from urine(14). The tissue activator is more stable and is very strongly attached to structural proteins. It is known, furthermore, that fibrinolytic activity develops very suddenly and appears overall in the blood(1). Therefore, the blood activator is probably not identical with the tissue activator. There is reason to assume that the blood activator develops from a precursor, a proactivator, which apparently is present in large amounts in human blood.

Obviously, the determination of fibrinolytic activity yields different results with different substrates. A substrate free from plasminogen reacts only with the enzyme proper (plasmin). In a fibrinolytic method, performed as is normally the case, the plasminogen in the substrate may be activated by activators present in the active samples. Therefore, the total activity observed may be much larger than that corresponding to the content of plasmin in the sample. This makes a reconsideration of a large part of the existing literature in this field necessary. *Macfarlane and Pilling*(15) diluted active human globulin with *human fibrinogen containing plasminogen* and lysis was obtained in high dilutions. These large activities do not conform with the low spontaneous activities (plasmin) found in human blood with casein(8). *Lewis and Ferguson* have used bovine fibrin as a substrate(13). This substrate contains plasminogen and does not permit a distinction between activator and plasmin. Our recent experiments suggest that it is an activator and not an enzyme, which is formed spontaneously in their cited experiment.

Summary. 1. Fibrinolytically active blood, as obtained from the living and dead human organism, contains a plasminogen activator. The amount of this activator exceeds many times that needed for the complete activation of the plasminogen present in human blood. 2. The plasminogen activator in blood differs from the tissue activator, fibrinokinase, and is probably formed from a precursor contained in the blood.

1. Macfarlane, R. G., and Biggs, R., *Blood*, 1948, v3, 1167.

2. Bennike, T., and Müllertz, S., *Acta Haematologica*, 1952, v8, 147.

3. Mole, R. H., *J. Path. Bact.*, 1948, v60, 413.

4. Müllertz, S., *Acta Physiol. Scand.*, 1952, v27, 265.

5. ———, *Acta Physiol. Scand.*, 1953, in press.

6. Astrup, T., and Müllertz, S., *Arch. Biochem. Biophys.*, 1952, v40, 346.

7. Lassen, M., *Acta Physiol. Scand.*, 1952, v27, 371.
8. Cliffton, E. E., and Downie, G. R., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 559.
9. Müllertz, S., *Acta Physiol. Scand.*, 1952, v26, 174.
10. Astrup, T., and Permin, P., *Nature*, 1947, v159, 681.
11. Astrup, T., *Biochem. J.*, 1951, v50, 5; Lewis, J. H., and Ferguson, J. H., *J. Clin. Invest.*, 1950, v29, 1059.
12. Schmitz, A., *Zeitschr. Physiol. Chem.*, 1936, v244, 89.
13. Lewis, J. H., and Ferguson, J. H., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 184.
14. Astrup, T., and Sterndorff, I., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 675.
15. Macfarlane, R. G., and Pilling, J., *Lancet*, 1946, v251, 562.

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Effect of Chlorides and Heat on Extraction and Activity of Sheep Pituitary Gonadotrophin.* (20098)

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Aqueous solutions of organic and inorganic compounds, and also water have been used for the extraction of pituitary gonadotrophin(s)(1-5). The concentration and, particularly, the separation in high yields of two biologically pure gonadotrophins from these extracts have been difficult to accomplish. Presumably this is due in part to the association of the gonadotrophins (FSH and LH) with inert material and with each other. On the basis of this assumption attempts were made to dissociate these hormones by extracting them with saturated solutions of inorganic chlorides, concentrated solutions of sucrose and urea, and water at a temperature just below that which would cause inactivation. Although separation of the gonadotrophins was not accomplished, they were concentrated by simple procedures and information was obtained relative to their stability to heat and their augmentation by concentrated salt solutions.

Methods and materials. The solvents used for extracting the dry sheep pituitary tissue were solutions of inorganic chlorides saturated at room temperature, 0.88 M sucrose, 6.6 M urea and water. The amounts of pituitary tissue extracted ranged from 2 to 100 g. Extractions with sodium chloride were made at 25, 60, 70, 80 and 90°C. Extractions with the other solutions were done at 70°C. The pituitary powder was mixed in the ratio of 1 g of dry tissue to 10 ml of solution, placed in a water bath regulated to the desired temperature, stirred for one hour, centrifuged and the residue reextracted in the same way. The two extracts were combined and designated as extract E. A third treatment with NaCl solution extracted little activity. On the basis of this, the residues that remained after two extractions with the other solvents were not studied. *The E extracts* were dialyzed to remove the chlorides and other substances used for extraction. This caused an inactive precipitate to form which was removed by centrifugation to give the dialyzed soluble fraction A which was adjusted to pH 4. The precipitate was removed by centrifugation and designated as fraction C, and the pH 4 soluble fraction (B) was concentrated by lyophilizing. This concentrated extract was dialyzed, then centrifuged to remove any insoluble material present and lyophilized to dryness to give fraction D. Fraction B was also recovered by precipitation with alcohol or acetone and dried by lyophilization. The *extracts and fractions* were assayed for gonadotrophic hormone activity by the use of 21-day-old female rats of the Holtzman-Rolfsmeyer strain. The volumes of the fractions were adjusted with

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