status of any tumor of a given size.

Water soluble glycoproteins of tumor connective tissue show distinct increases as compared with normal connective tissue in Swiss and C_3H mice implanted with various tumors (Table II). Both sugar and protein (tyrosine) values are elevated. In some cases the sugar/tyrosine ratios show wide fluctuations from the expected values; the reason for this has not been explained.

Summary and conclusions. Serum glycoproteins are increased in mice bearing transplantable tumors. Water soluble glycoproteins are increased in the connective tissue bordering and abutting on the tumor. These findings support the thesis that increased circulating glycoproteins arise from the ground substance of the connective tissue at the site of invasive growth by a process of depolymerization, whereby smaller, water soluble and diffusible glycoprotein moieties are produced.

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Quantitative Estimation of Plasma Accelerator-Globulin. (18152)

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In a previous publication(1), a method was described whereby oxalated bovine plasma could be rendered free from Ac-globulin. This solution ($HgCl_2$-plasma), containing 50-60 Iowa units/ml of prothrombin, has provided a stable, reliable source of prothrombin for the quantitative estimation of Ac-globulin in plasma. The method of assay, based upon the use of three variables—conversion rate, thrombin yield, and onset of thrombin formation, is a modification of the method of Ware and Seegers(2).

Method. 1. Human and dog whole blood is collected in 1.85% potassium oxalate; 7 parts of blood to 1 part of anticoagulant are used. For rats, 1 ml of blood is taken into a syringe containing 0.23 ml of 1.85% potassium oxalate. The blood is centrifuged at 5000 r.p.m. for 15 minutes, and the hematocrit and oxalate correction factor recorded.

2. Determine the amount of prothrombin in the plasma to be tested for Ac-globulin using the modified two-stage technic(3,4).

3. Into a chemically clean glass test tube (120 x 13 mm), place 4 ml of physiologic saline.

4. Add $HgCl_2$-plasma in an amount such that the prothrombin in the $HgCl_2$-plasma, plus the amount of prothrombin in the plasma to be assayed for Ac-globulin equals 50 units.

5. To the diluted $HgCl_2$-plasma, add 0.08 ml of the human plasma (0.008 ml for dog, rat, and beef plasma) to be assayed.

6. Add physiologic saline to the tube to bring the total volume to 5 ml. The prothrombin concentration is now 10 units/ml. Swirl tube to insure thorough mixing of components.

7. To 1.5 ml of incubation mixture,* add 0.5 ml of contents of the dilution tube (step 6).

8. Incubate at 28°C for 5, 8, 11, 14, 17, 23, 26, and 30 minutes. At the end of each of these time periods, add 0.2 ml of the contents of the incubation tube (step 7) to 0.05 ml of a 1.0% solution of Armour bovine fibrinogen, and record the clotting time.

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3. Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, v114, 667.

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* Incubation mixture is prepared as follows: calcium saline (1% $CaCl_2$ in physiologic saline), 3 parts; 15% acacia (containing 0.04% calcium by weight), 2 parts; imidazole buffer (pH 7.2), 1 part. To 10 ml of this solution is added 5 ml of heated crude lung thromboplastin.

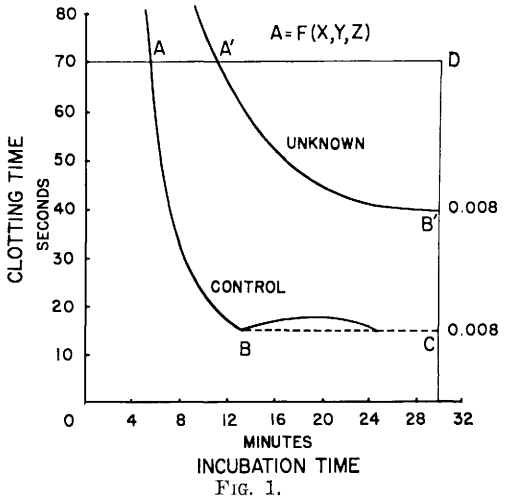

FIG. 1.

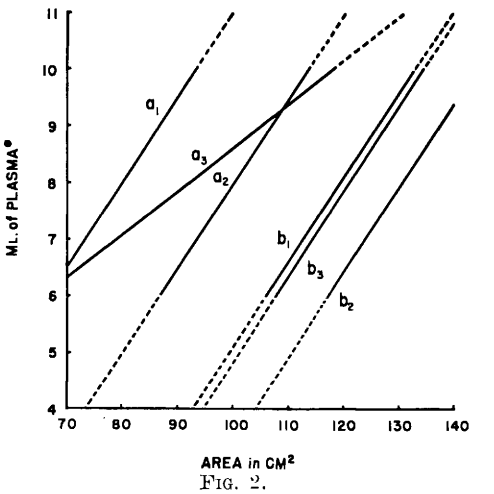

FIG. 2.

*ml of plasma × 10-2 human; × 10-3 dog, rat. a—undefibrinated, b—defibrinated plasma; 1—human, 2—dog, 3—rat. Throughout this paper, defibrinated plasma is that which has been treated with 10 units/ml of thrombin and the fibrin clot removed after standing 20 min.

9. On arithmetic graph paper (Fig. 1), plot the activation curve: clotting time in seconds (ordinate) against the incubation time in minutes (abscissa). Draw a vertical straight line through the 30 minute incubation time, and a horizontal straight line through the 70 second clotting time. From the point of maximal thrombin yield on the activation curve (B), draw a straight horizontal line to the 30 minute vertical line.

10. Measure the area in cm^2, preferably with a planimeter, bounded by the activation curve, and the horizontal and vertical straight lines as set forth above (area A, B, C, D). The area derived from the plasma to be assayed for Ac-globulin activity and that derived from the same volume of a normal control plasma is measured.

11. By referring the areas to a standard reference curve (Fig. 2), the Ac-globulin activity can be expressed in percent of normal. Through a point determined by the volume of normal control plasma used in the assay and its corresponding derived area, draw a line parallel to those on the reference curve. Using this line, mark the point on it at which the area obtained with the unknown plasma crosses it. The corresponding volume of plasma (ordinate) is therefore that amount which contains Ac-globulin activity equivalent to that which would have been obtained with the normal control plasma. Therefore:

$$\frac{\text{derived ml of unknown plasma} \times \text{ocf}\dagger}{\text{actual ml of plasma used} \times \text{ocf of normal control}} \times 100 = \%\ \text{Ac-globulin activity.}$$

12. The accurate area range for calculation of human Ac-globulin activity requires the use of from 0.06 to 0.10 ml of plasma. The calculated area of an unknown plasma must be such that the corresponding volume of plasma falls within this range. To preclude the necessity of determining the actual area in order to find the proper range, however, recourse may be had to a range finder graph (Fig. 3). The area is plotted against the clotting time at a constant incubation time. For example, if 0.08 ml of an unknown human plasma gives a clotting time of approximately 70 seconds after an incubation time of 12 minutes, the corresponding area would be approximately 80 cm^2 which is in the correct range. If however, the clotting time were 100 seconds, the corresponding area would be 60 cm^2, and therefore outside of the range. Redilution of plasma would be necessary.

The thromboplastin used in the present quantitative study is the initial fraction of the crude saline extract prepared from fresh beef lung(5). The extract is diluted 1-10, placed in a waterbath at 56°C for 30 minutes,

† Oxalate correction factor.

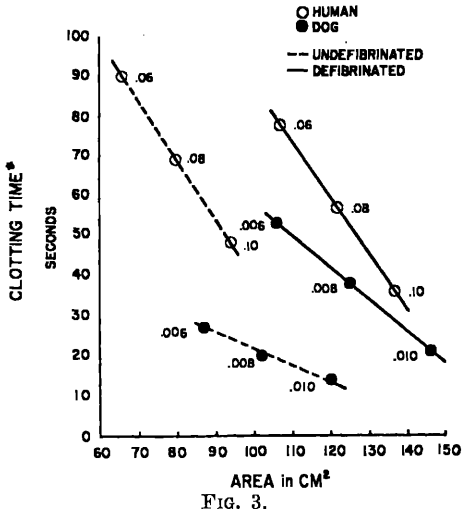

FIG. 3.

*Clotting time at 12 min. incubation using undefibrinated plasma; at 6 min. incubation using defibrinated plasma.

and centrifuged for 10 minutes at 5000 r.p.m. The resulting solution is an easily prepared, clear, highly active thromboplastin without trace of prothrombin or Ac-globulin. It is stable at least 18 months when stored at –40°C. The use of this preparation was occasioned by the presence of small but significant amounts of Ac-globulin in some batches of purified thromboplastin prepared after the method of Chargaff *et al.*(6).

Discussion. The Ac-globulin activity in any given plasma is manifested as a function of 3 variables: (1) the time when prothrombin conversion begins, (2) the rate of prothrombin conversion, and (3) the yield of thrombin. Thus the area, circumscribed by the activation curve and by straight lines through a constant arbitrarily selected point on each axis (Fig. 1), is a measure of Ac-globulin activity which includes all three variables. A normal control plasma always should be assayed to determine the intercept on the reference curve. Although each or any combination of the three variables theoretically could be used as a criterion for the assay of Ac-globulin activity, our quantitative data indicate that by using all 3 variables, a greater degree of accuracy is obtained.

The data used in the formulation of the reference curves for each species are based upon composite Ac-globulin assays of normal plasma from 33 humans, 15 dogs and 12 rats respectively. Four human and all rat assays were done on pooled plasmas of 4-5 each. The curves shown in Fig. 2 represent the linear component of sigmoid curves within a range of from 6 to 10 x 10^{-2} ml for human, and from 6 to 10 x 10^{-3} ml for dog and rat plasmas. Within this range, the slopes are relatively constant. Sixty-seven per cent of the human slopes varied less than 10%; 88% varied less than 15%; 87% of the dog slopes, and 92% of the rat slopes varied less than 10%. The average slope for human and dog non-defibrinated plasma and for human, dog, and rat defibrinated plasma was 1.5. Non-defibrinated rat plasma gave a slope of 0.75. With rat plasma, however, twice as much oxalate was used to prevent clotting as compared with other species. When the same amount of oxalate is used, the slope approaches 1.5, but clotting of plasma may occur. When 4 times as much oxalate is used, the slope approaches 0.37. Thus it would appear that the slope of the curves is at least partially dependent upon the amount of oxalate. For this reason, the amount of oxalate should be controlled carefully.

As the activity of Ac-globulin increases or decreases, the position of the sigmoid curves varies on coordinate paper. If the calculated areas remain within the range of 70 to 150 cm^2, however, the slopes of the linear segments of the sigmoid curves remain constant enough so that these segments may be used as the basis for formulating the reference curves. As this range is exceeded, the slopes of the curves tend to deviate significantly. Of even greater importance than the area range, however, is the volume range. Irrespective of what the area range may be, it is essential that the volumes of plasma used vary from 6 to 10 x 10^{-2} for human, and from 6 to 10 x 10^{-3} for dogs and rats. From experience, we have found that below this range, the slopes vary appreciably, and that the curves deviate significantly from a

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6. Chargaff, E., Moore, D. H., and Bendich, A., *J. Biol. Chem.*, 1942, v145, 593.

linear function. We attribute this to an obscure factor(s) which in some manner probably interferes with the activity of Ac-globulin. Our data suggest that this factor may be fibrinogen concentration. In a previous publication(7) and in quantitative data to be published, the importance of fibrinogen on Ac-globulin activity has been stressed. In constructing the reference curves from the normal control plasmas, it was observed that the maximum deviation of areas as well as the variation of slopes became progressively less as the accurate volume range was approached. Moreover, the maximum deviation of areas and slopes was decidedly less with defibrinated than with undefibrinated plasma. When 1 to 5 x 10^{-2} ml of human plasma were used, the slopes of the lines derived from undefibrinated plasma were consistently less than those from defibrinated plasma. As the volume of plasma was increased to the accurate range, the slopes of the undefibrinated and of the defibrinated plasma approximated each other. Thus, the observations would indicate that fibrinogen alters the Ac-globulin activity and therefore the slopes of the reference curves, and that Ac-globulin assays can be done more accurately on defibrinated plasma.

Variables, such as temperature, reactivity of the biologic reagents etc. which affect other assay methods in blood coagulation, likewise exert their influence on the assay of Ac-globulin activity. The influence of these variables is manifested as a shift in intercept of the reference control curves from day to day. These variables do not alter significantly the slope of the reference curves. The existence of such variables, however, does demand that the intercept be established each day by the assay of Ac-globulin activity on normal control plasma.

In the assay of Ac-globulin activity, the prothrombin concentration is adjusted so that 2 units rather than 1 unit are present in the clotting tube. As a result, a smaller volume of plasma is required for the estimation of Ac-globulin activity, and the effect of antithrombin on the conversion rate and thrombin yield is correspondingly decreased.

Summary. A method for the quantitative estimation of Ac-globulin in plasma has been described. The method is based upon the use of three variables—conversion rate, thrombin yield, and onset of thrombin formation. With the use of all three variables combined, a greater degree of accuracy can be obtained.

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Effects of B Vitamins and Liver on Growth of Immature Rats Maintained at Low Temperatures.* (18153)

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Available data indicate that requirements for a number of nutrients are markedly increased under conditions of low environmental temperature. An increased requirement for thiamine(1,2), riboflavin(3), pyridoxine(4),

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