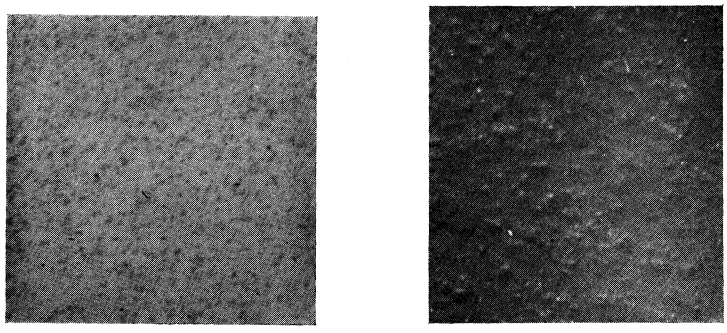

FIG. 1.
Electron micrographs and shadowgraphs of an infected mousebrain suspension containing 3.9×10^{-14} g nitrogen per LD_{50}. Magnification 30,000.

ticle dimensions could be made by measuring the shadow width and shadow length. The shadow length should be 5 times the diameter.

In both micrographs there is an indication for a slight asymmetry of the particles. In measuring the diameter of the particles a minimum dimension of 10 mμ and a maximum of 20 mμ with an average of about 12 mμ was found.†

These micrographs confirm the findings of Loring, Marton and Schwerdt[2] of the absence of thread-like particles[3] and show also essentially the same shape and size of particles as described by these authors.

Summary. Electronmicroscopic studies of a preparation of purified MM Poliomyelitis virus show an uniformity of particle size in the range from 10 to 20 mμ with an average diameter of about 12 mμ.

† These measurements would indicate a molecular weight of the order of 500,000 to 1,000,000.

2 Loring, H. S., Marton, L., and Schwerdt, C. E., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 291.

3 Gard, S., *Acta Med. Scand.*, Suppl., 1943, **143**, 173.

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Serum and Plasma Antithrombin.*

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For years it has been known that blood has the ability to inactivate added, as well as spontaneously formed, thrombin. Rettger[1] attributed this to the plasma proteins as a specific function; Lenggenhager[2] related the activity to the albumin fraction. Smith's[3] group has suggested that the *rate* of thrombin destruction varies with the heparin concentration and that the absolute *amount* is limited by the concentration of heparin "cofactor." Wilson[4] developed a method for estimating antithrombin by so diluting plasma

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1 Rettger, L. J., *Am. J. Physiol.*, 1909, **24**, 406.

2 Lenggenhager, Karl, *Helv. med. Acta*, 1935, **1**, 527.

3 Seegers, W. H., Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Science*, 1942, **96**, 300.

4 Wilson, S. J., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 676.

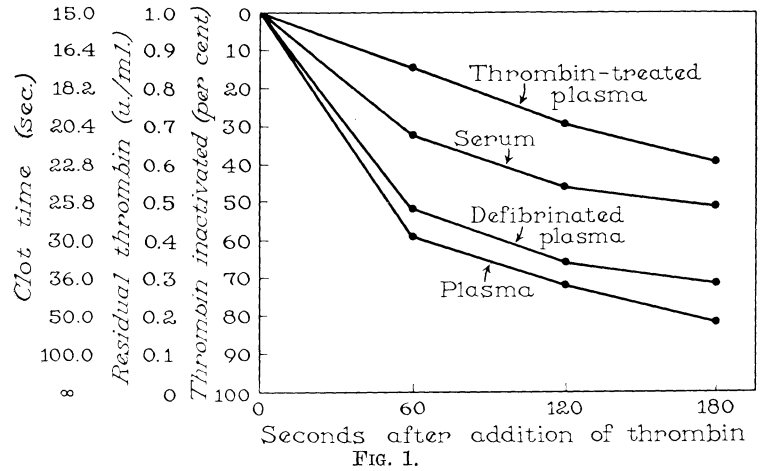

FIG. 1.
Deterioration of thrombin added to diluted serum or plasma.

that it inactivates 1 unit of thrombin in 4 minutes. Astrup and Darling,[5] adding an excess of thrombin to a small amount of plasma, await maximal inactivation before measuring the residue. By both methods no difference in the antithrombic activity of serum and plasma was detected, in spite of the fact that serum inactivates about 300 thrombin units per milliliter in its development.

In an attempt to increase the sensitivity of measurement of thrombin inactivation, the following method was devised. Serum or oxalated plasma is diluted tenfold with buffered saline solution (1 part isosmotic imidazole buffer,[6] 9 parts 0.9% sodium chloride). To 1.0 ml of this solution is added 0.1 ml of thrombin (11 units per milliliter), to make a final thrombin concentration of 1 unit per milliliter. In the case of plasma, fibrin begins to form in 20 to 30 seconds; sharp agitation of the tube clumps the fibrin and prevents its obscuring the final step. Exactly 60 seconds after the addition of thrombin, and in some cases at intervals thereafter, 0.4 ml of the solution is added to 0.1 ml of 0.5% buffered fibrinogen solution, and the coagulation time is determined. The amount of residual thrombin is calculated by comparison of the clot times with the times found for dilutions of thrombin with buffered fibrinogen solutions (Fig. 1).

Tests were performed at 24° to 26°C. Parke-Davis beef thrombin and Armour's Fraction I (fibrinogen) were routinely employed, but were checked against homologous preparations, with negligible variation in the results.

By the use of this method it was found that oxalated plasma (diluted 1:10) from 12 normal dogs inactivated about 60% (40 to 82) of the added thrombin in 60 seconds (Fig. 1). Serum (diluted 1:10) from the same blood samples inactivated about 25% (14 to 38) in 60 seconds. Oxalation of the serum, or preliminary defibrination of the plasma by the addition of only a fraction of a unit of thrombin, altered the results only slightly. The significance of the amount of thrombin deterioration may be estimated from this experiment: a 1:2 dilution of pooled normal dog serum inactivated 81% of added thrombin in 1 minute; 1:3 serum, 67%; 1:4 serum, 50%; 1:5 serum, 39%; 1:6.7 serum, 28%; 1:10 serum, 23%; 1:20 serum, 10%; 1:40 serum, 7%; and 1:100 serum, trace.

To examine the serum-plasma difference further, dicumarol was administered intravenously to dogs; blood samples were collected periodically. Along with each sample preserved by oxalate, another specimen was

[5] Astrup, Tage, and Darling, Sven, *Acta physiol. Scandinav.*, 1942, **4**, 293.

[6] Mertz, E. T., and Owen, C. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 204.

[7] Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667.

TABLE I.
Antithrombic Activity of Plasmas and Serums from Two Dicumarol-treated Dogs (A and B).

% prothrombin in plasma*		% thrombin deterioration in 60 seconds	
A	B	Plasma	Serum
100 (320 units per ml)		59	32
	100	61	36
	77	64	43
71		60	35
	66	67	38
52		68	45
	45	60	54
43		63	53
	33	66	58
31		68	56
	24	62	58

* Two-stage titration.[7]

TABLE II.
Antithrombic Activity of Plasma at Various Stages During Spontaneous Clotting.

Time after collection of blood, min	% prothrombin*	% thrombin deterioration in 60 sec.
Immediately	100 (305 units per ml)	61
3	68	53
5	52	48
7†	36	44
9†	21	40
11†	3	33
15†	1	33

* Two-stage titration.[7]
† Fibrinogen-free.

set aside for development of serum. Table I shows the antithrombic activity of comparable plasmas and serums from 2 dogs. If antithrombic determinations had been made on serums alone, the evidence would suggest more antithrombin in serums from the low prothrombin samples than in serums from the high prothrombin samples. From study of the oxalated plasmas alone, no difference was seen.

Somewhat similar results are noted in the following experiment (Table II). Fifty milliliters of normal dog blood were collected; at intervals 4.5 ml were added to 0.5 ml of 1.85% potassium oxalate. Clotting began at about the sixth minute; thus the last 4 specimens were fibrinogen-free. It would seem that serum has an antithrombic activity varying inversely with the amount of thrombin to which it has been exposed during clotting.

If this is correct, addition of sufficient thrombin to plasma should simulate the effect of spontaneous clotting. One milliliter of oxalated plasma was mixed with 1.0 ml of 500 units per milliliter thrombin. In 2 hours no thrombin was demonstrable. After appropriate dilution the antithrombic activity of this solution was less than that of control serum: thrombin-treated plasma, 14% inactivation in 60 seconds; serum, 30% (Fig. 1).

Summary. A method of estimating the rate of natural antithrombic activity is suggested. When this method was used, dog plasma, regardless of the prothrombin level after dicumarolization, seemed to exert about the same antithrombic activity. On the other hand, dog serum antithrombin tended to vary inversely with the prothrombin content of its original plasma, and serum obtained from blood containing little prothrombin was antithrombic to about the same extent as plasma. These observations suggest that antithrombic activity be determined on plasma unless due consideration is given to the antithrombic activity already performed by serum.