Typical results with each of 16 local anaesthetics alone and in conjunction with sulfapyridine are shown in Table I. It will be noted that each of the 7 p-aminobenzoic acid derivatives partially or completely blocked the bacteriostatic effect of the sulfapyridine; the blocking effect was reduced but not eliminated by substitution in the amino group or esterification of the carboxyl group of p-aminobenzoic acid. None of the 9 local anaesthetics not derived from p-aminobenzoic acid displayed any antagonism to the sulfapyridine. In these experiments, p-aminobenzoic acid could counteract the effect of approximately 200 to 500 molecular equivalents of sulfapyridine; from Table I the corresponding ratios for local anaesthetics nos. 2, 3, and 4 were, respectively, approximately 20, 40, and 2.

Each of the 3 p-aminobenzoic acid derivatives blocked the action of sulfathiazole; none of the non-p-aminobenzoic acid derivatives displayed any antagonism to sulfathiazole (Table II).

Summary. Each of 7 local anaesthetics derived from p-aminobenzoic acid partially or completely blocked the *in vitro* bacteriostatic effect of sulfapyridine upon a strain of *B. coli* grown in a simple medium. None of 9 local anaesthetics not derived from p-aminobenzoic acid displayed any antagonism to the sulfapyridine. Each of 3 local anaesthetics derived from p-aminobenzoic acid blocked the bacteriostatic effect of sulfathiazole on *B. coli* and *Staphylococcus aureus*. None of 3 local anaesthetics not derived from p-aminobenzoic acid displayed any antagonism to the sulfathiazole.

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The Assay *Recovery* of Prothrombin Added to Plasma.

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In the promulgation of current methods of prothrombin assay[1, 2] there has been some investigation of plasma dilutions but no direct experiments to show the ability to *recover* prothrombin added to plasma. This is important in connection with the possibility that

[1] Quick, A. J., *J. Am. Med. Assn.*, 1938, **110**, 1658.

[2] Brinkhous, K. M., *Medicine*, 1940, **19**, 329.

clot-inhibitors[3-6] may complicate the interpretation of the assay. It is recognized, for instance, that heparin interferes with prothrombin determinations[2] and heparin can act both as an antiprothrombin[6] and as an immediate antithrombin,[5] in the presence of the respective plasma co-factors. The natural antithrombins also include a progressive thrombinolytic factor.[4] As an example of the kind of interference which may be expected, it has recently been found that a series of prothrombin dilutions, subsequently activated with Ca^{++} and brain thromboplastin, gives more than the theoretical thrombin yield, as determined from the clotting-times for purified fibrinogen, using a similar dilution series of the same (full-strength) thrombin as the standard of reference.[6] In the absence of equivalence it is, of course, impossible to assay prothrombin *both* in terms of an arbitrarily fixed clotting-time *and* a definite thrombin dilution value. Inherent in the clinical tests is a false assumption that inhibitors do not influence the results obtained. Actually, there must be a rôle for the natural inhibitors of the types mentioned. The Iowa workers[2] recognized only the progressive antithrombins and succeeded in controlling these by dilution. It is an unproved assumption that immediate antithrombins and antiprothrombins do not interfere with the prothrombin assay. In the following experiment, in which a prepared prothrombin is assayed in the presence of plasma, these problems are readily brought to test.

Assay of Prothrombin and Plasma. Method. A Howell-type prothrombin solution[7] is converted into thrombin by the use of 1/20 vol. N/10 $CaCl_2$ and a similar amount of saline emulsion of frozen dog brain (thromboplastin). The thrombic mixture is held at 10°C and 0.5 cc samples tested *(A)* at the cited intervals, by timing the clotting after adding to 1.0 cc fibrinogen (temp. = 37°C, pH = 7.5). Similar tests are made on citrated dog plasma which has been heated to 56°C, to defibrinate, and diluted with 0.9% NaCl. Data are given for several dilutions of prothrombin and plasma and mixtures of both.

Either the full-strength thrombin or that from the diluted prothrombin (activated) can be used as the standard of reference. Serial dilutions of the thrombin are made and the clotting-times for fibrinogen, under the above uniform conditions, are used for

3 Ferguson, J. H., *Am. J. Physiol.*, 1940, **130**, 759.

4 Glazko, A. J., and Ferguson, J. H., *J. Gen. Physiol.*, 1940, **24**, 169.

5 Glazko, A. J., and Ferguson, J. H., *Am. J. Physiol.*, 1941 (in press).

6 Ferguson, J. H., and Glazko, A. J., *Am. J. Physiol.*, 1941 (in press).

7 Ferguson, J. H., *J. Lab. Clin. Med.*, 1938, **24**, 273.

the reference curve *(B)*. The *optimal* clotting-times for the prothrombin and plasma activation series are referred to the thrombin dilution curve, preferably plotted as reciprocal C. T. [r = 1000 ÷ C. T. (seconds)] against percentage concentration (in terms of original strength). This gives the "recovery" values cited in *C*.

Data (Table I). *Exp. 2.* The increase (to 16%) over the theoretical (10%) is due to dilution of natural antiprothrombin, according to the interpretation previously submitted.[6] *Exp. 3, 4, 6.* In these cases, the 5-minute test is already past the optimum potency because of thrombic destruction by progressive (serum-) antithrombin.[4] The true equivalence, therefore, is greater than the figures obtained from the clotting-times.

Exp. 6. The apparent inactivation rate is slower than in Exp. 4, presumably due to the greater thrombin yield because of the added prothrombin. Even allowing for the uncertainty due to the presence of progressive antithrombin, there is an excess over the theoretical which points to a natural antiprothrombin in the plasma. *Exp. 7, 8.* Here the plasma dilution (50-100 x) is sufficient to ensure a stable thrombin (*i. e.*, practically no progressive antithrombin). When the antiprothrombic factor is allowed for, by using the referred (corrected) thrombin equivalent, the "recovery" values check perfectly. They are practically as good, *viz.* 116 (117) and 85 (84.5) if the

TABLE I.

"Recovery" of Prothrombin Added to Diluted Plasmas.

A. Activation Curves (10°C). Clotting-times (sec.) at 37°C. pH = 7.5.

T	Source of thrombin	5′	15′	30′	60′
1.	Prothrombin (1:1)	*9″*	9″	9″	9″
2.	" (1:10)	120″	35″	*30″*	33″
3.	*Plasma (1:1)	*9″*	25″	125″	1800″
4.	* " (1:10)	*12″*	15″	22″	35″
5.	* " (1:100)	235″	*40″*	40″	40″
6.	* " (1:10) + Pro. (1:10)	*12″*	15″	18″	28″
7.	* " (1:50) + Pro. (1:5)	80″	*34″*	34″	34″
8.	* " (1:100) + Pro. (1:10)	22″	*21″*	21″	21″

* Plasma defibrinated by heating (56°C). Equal volumes of plasma and prothrombin (Pro.) were mixed in *6, 7, 8.*

B. Thrombin (T_1) Dilution Curve = standard of reference.

Dilution	1:1	1:2	1:4	1:8	1:16	1:32	1:64
Clotting-time	9″	14″	22″	35″	52″	80″	113″

C. Prothrombin "Recovery" (Thrombin *Percentage*): Theoretical values in parentheses.

	1.	*2.*	3.	4.	*5.*	6.	*7.*	*8.*
%	100	16(10)	>100	>65	10	>65(>40)	26(26)	13(13)

1:10 (pro) thrombin is used as the starting point for the standard of reference. On another occasion, the prothrombin "recovery" (theoretical in parenthesis) in 2 similar experiments gave (a) 80 (85), (b) 70.5 (70).

Comment. In spite of the demonstrated possibilities of interference by clot inhibitors, the ability to obtain such excellent prothrombin *recovery* shows that these inhibitors are under control, notwithstanding the empirical nature of the assay method. The data confirm the Iowa workers' observation[2] that dilution removes the effects of progressive antithrombins. Immediate antithrombins[5] and antiprothrombins[6] do not interfere, although they are undoubtedly present. The latter, indeed, are demonstrated by analysis of the data. Consideration of the reference standard suggests an explanation. The comparisons are made on the basis of *clotting-times.* Thus the arbitrary thrombin "percentage" assumes a new significance in that it is automatically corrected for differences in the equivalence of thrombin and prothrombin. The only remarkable point is that the same correction seems to hold for diluted plasma and the prothrombin, which was prepared some weeks previously from another animal. The values obtained, of course, are not strictly measures of prothrombin in terms of thrombin but rather in terms which tacitly include variables imposed by the inhibitors present in both. It is justifiable to conclude that the natural inhibitors do not vary under the circumstances investigated. However, this might not hold under other conditions and it is conceivable that certain clinical anomalies of "prothrombin" clotting-times may reside, not in the prothrombin *per se,* but in the associated inhibitor variables. These conditions obtain in heparinized plasma and it is highly improbable, on the basis of recently published studies[5, 6] that they can be overcome by simple dilution or the excess of thromboplastin, upon which the modern prothrombin methods essentially depend.