that enhancement of twitch tension produces a certain pattern of change in efficiency. The twitches directly following a tetanus are greatest and the decay has a linear relationship. While twitch responses occurring shortly after a period of tetanus show characteristic changes in efficiency, they are not large in comparison with the changes in tension. Enhancement, as measured by twitch tension, is at first associated with a small decrease in efficiency and then a small increase in efficiency with a return to pre-tetanic levels. On the other hand, when the post-tetanic twitch is depressed, the efficiency is temporarily increased.

Post-tetanic twitch responses may show enhancement or depression with tensions of 50% greater to 50% less than the pre-tetanic values. The initial heat developed with each twitch varies with the tension so that the efficiency of contraction is not markedly altered, and cannot account for the changes in tension.

Summary. 1. The twitch tension and the initial heat developed was determined in frog double sartorius preparations during the period following tetanic stimulation. 2. When enhancement occurs there is at first a small decrease in efficiency, followed by a rise slightly above the original level, with return to the pre-tetanic level, as the enhancement subsides. 3. When post-tetanic depression occurs there is a small increase in efficiency followed by a gradual return to the pre-tetanic value. 4. The efficiency changes are small in comparison with the changes in twitch tension, and thus cannot account for the changes in the mechanical response.

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Estimation of the Anticephalin Activity of Whole Blood.

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The demonstration of anticephalin* activity in plasma is rendered difficult by a number of interfering factors. The time and manipulations involved in the separation of the plasma, the addition of anticoagulants and the subsequently required recalcification, the inactivating effect of contact with glass or similar surfaces are among some of the factors that interfere with the accurate testing of this activity in plasma.[1,3] Since anticephalin manifests itself chiefly during the initial stages of coagulation (before the conversion of prothrombin), any imperfections in the collection of blood or in its mixture with the anticoagulant will affect the results. It would seem desirable, therefore, that the activity be estimated without the use of anticoagulants, and as soon as the blood is collected.

As shown elsewhere[4] the response of a given plasma to activation by cephalin, the magnitude of the clot-decelerating effect of incubation of the plasma with cephalin, and the difference between the rate of coagulation of plasma on glass and on paraffin or lusteroid

* The term *cephalin* is used to designate the alcohol-insoluble lipid fraction extracted with ether from acetone-dried human brain.[1] Cephalin suspensions so obtained are obviously not pure. Most indications point to a cephalin as the active fraction of the thromboplastic lipoprotein[2] and it may so be considered until convincing evidence to the contrary is available. The term *anticephalin* is intended to designate that activity of plasma directed against the thromboplastic action of cephalin.

[1] Tocantins, L. M., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 94.

[2] Quick, A. J., *Hemorrhagic Diseases and the Physiology of Hemostasis*, 1942, p. 68.

[3] Tocantins, L. M., *Am. J. Physiol.*, 1943, **139**, 265.

[4] Tocantins, L. M., *Am. J. Physiol.*, in press.

surfaces appear to maintain a certain correlation, when other factors (prothrombin, antithrombin) remain equal or under control. When cephalin is added to plasmas with low anticephalin activity, clotting occurs more rapidly than normal, and at about the same rate in glass or lusteroid tubes. Conversely, in the presence of excessive anticephalin activity (*e.g.*, hemophilic plasma), the clot-accelerating effect of cephalin is lessened, and the difference between the rate at which coagulation proceeds in glass as compared with paraffin-coated or lusteroid† tubes is magnified.[4] When dealing with intact whole blood it is manifestly not feasible to estimate anticephalin activity by incubating blood with the lipid. It appears, however, that some dependence may be placed simply on the response of the blood to the cephalin and on a comparison between its rate of coagulation on glass and other surfaces.

Methods. Human venous blood is collected into an oiled syringe observing the especial precautions previously outlined.[3] Since the addition of cephalin must take place at the bedside, as soon as the blood is collected, tubes (13 mm i.d. x 60 mm length, one of pyrex glass, the other of lusteroid or glass coated with paraffin) containing the measured amount of cephalin suspension are held ready immersed in water at 38°C, in a wide neck thermos bottle. One cc of blood is mixed with 0.1 cc of the cephalin suspension and the time required for coagulation measured with a stopwatch. The clotting time of plain blood in the two types of tube is also measured, simultaneously. When citrated as well as intact blood are desired, an assistant is necessary to insure expeditiousness in handling the collection of blood into 2 syringes, the first one holding the anticoagulant (1 part of a 0.129 M solution of trisodium citrate to 9 parts of blood), the second reserved for plain blood.

Plasma anticephalin activity is estimated by noting the delay in the clotting time induced by incubating (38°C) the citrated plasma with cephalin for 20 minutes before recalcification. Both glass and lusteroid tubes have been used. In view of the disturbing effects of contact with glass,[4] the results obtained on lusteroid surfaces are considered to represent the actual extent of this activity. Methods for preparing the cephalin suspension and other technical details are described elsewhere.[1,3,4]

Results. Venous blood to which cephalin is added takes about twice as long to clot in lusteroid as in glass tubes (Table I). The clot delaying effect of incubation of the citrated plasma with cephalin is, however, more than 4 times as great in lusteroid tubes, a fact which has led us to use these or similar tubes almost exclusively in testing anticephalin activity. The prolongation is not due to changes in the plasma brought about by the incubation *per se*. When normal citrated plasma (0.3 cc) was allowed to stand for 20 minutes in a stoppered glass tube at 38°C, its mean clotting time (11 samples) upon recalcification and addition of cephalin was 81.4 seconds in contrast with the mean time of 126.1 seconds when the plasma had been previously incubated with cephalin before recalcification. When lusteroid tubes were employed, the respective times (11 samples) were as follows: Plasma alone, at 38°C for 20 minutes before adding $CaCl_2$ and cephalin = 237 seconds; plasma incubated with cephalin for 20 minutes before recalcification = 393.8 seconds.

The response of a given plasma to activation by cephalin is closely correlated with its anticephalin activity (Fig. 1, Chart 1). Plasmas exhibiting the longest clotting times after the addition of cephalin (*e.g.* hemophilic) are also those best able to reduce the clot accelerating action of the lipid after incubation with it. An even higher positive correlation exists between plasma anticephalin activity (lusteroid tubes) and the cephalin clotting time of blood in a lusteroid tube (Fig. 1, Chart 2). Moreover, the longer is the cephalin clotting time of blood in a lusteroid tube the greater is the difference between that time, and that measured in a glass tube (Fig. 1, Chart 3). This appears to indicate that whatever influences this difference, influences in a similar fashion the clot-accelerating action of cephalin on the blood. Actually the difference between the reaction of the blood to

† Plastic cellulose derivative manufactured by the Lusteroid Co., South Orange, New Jersey.

TABLE I.

Clotting Time in Glass and Lusteroid Tubes of Venous Blood of Normal Young Men (Ages 20-26), with and without Addition of Cephalin. Clotting Time of Normal Plasma with and without Incubation with Cephalin.

	Blood				Plasma			
					Cephalin clotting time‡			
	Clotting time*		Cephalin clotting time†		Glass		Luster.	
	Glass	Lust.	Glass	Lust.	0′ inc.	20′ inc.	0′ inc.	20′ inc.
Mean (sec)	615.6	1953	124.1	244.6	91.5	114.8	194.6	362
Stand. deviation (sec)	80.9	374.6	13.2	38.6	13.3	15.4	41	109
Maximum (sec)	780	2700	163	300	115	158	297	730
Minimum (sec)	480	1320	101	166	72	85	122	202
No. determ.	26	26	26	26	33	33	34	34
No. subjects	19	19	19	19	26	26	26	26

* 1.0 cc venous blood in 13 mm i.d. tubes at 38°C.

† 1.0 cc venous blood + 0.1 cc cephalin, in 13 mm i.d. tubes at 38°C.

‡ 0.3 cc plasma, 0.1 cc cephalin (0′ or 20′ incubation), 0.1 cc 0.074 M $CaCl_2$, in 13 mm i.d. tubes at 38° C.

cephalin in the two tubes has yielded a high correlation with the anticephalin activity of plasma (Fig. 1, Chart 4). On the basis of the above relationships, the cephalin clotting time of blood in lusteroid, paraffin, or similarly coated tubes and the difference between it and that measured in a glass tube may be used with advantage in estimating anticephalin activity. Such relationships, however, apply only when the prothrombin content is near normal, and there is no excess of antithrombin. A naturally occurring excess of antithrombin in blood is rare, but the possible existence of a low prothrombin makes a quantitative determination of this variable necessary. The regression equation correlating cephalin clotting time of blood with plasma anticephalin activity (Fig. 1, Chart 2) must, as further data accumulate, include a correction for the amount of prothrombin in the blood.

The cephalin clotting time of blood is also significantly correlated with the rate of coagulation of plain blood (without cephalin) (Fig. 1, Chart 5). Bloods which respond slowly to cephalin also show the greatest difference in their rate of coagulation between glass and lusteroid tubes (Fig. 1, Chart 6). This may indicate that, unlike certain thromboplastic agents, cephalin acts as the natural clot accelerators of the blood (platelets, leucocytes) and is, like them, vulnerable to their natural antagonists.

In Table II are given examples of these determinations in patients with hemorrhagic disorders. After hemorrhage (subjects 1 and 2) there is hypercoagulability of the blood, shortening of the cephalin clotting time of blood and plasma (especially evident in lusteroid or paraffin coated tubes), diminution in plasma anticephalin activity and reduction or even elimination of the difference in the rate of coagulation of blood and plasma between the two tubes. The relative number of platelets does not seem to influence the behavior of the blood towards the cephalin in the two tubes. The presence of a thrombopenia *per se* (subject 3, not bleeding at the time of observation) likewise leads to no significant changes in the blood or plasma cephalin clotting time. In hemophilics (*e.g.*, subject 4) the blood and plasma respond slowly to cephalin, a wide difference is observed between the rate of coagulation in the two tubes, and the plasma anticephalin activity is high. The clotting times of hemophilic blood with and without addition of cephalin are longer than those of hypoprothrombinemic (subject 5) or heparinized (subject 6) blood. Plasmas separated from hypoprothrombinemic and heparinized blood often display an increase in anticephalin activity, the magnitude of which depends on the degree of hypoprothrombinemia or the amount of heparin present. The exaggeration of anticephalin activity in moderately heparinized blood manifests itself, however, principally when testing

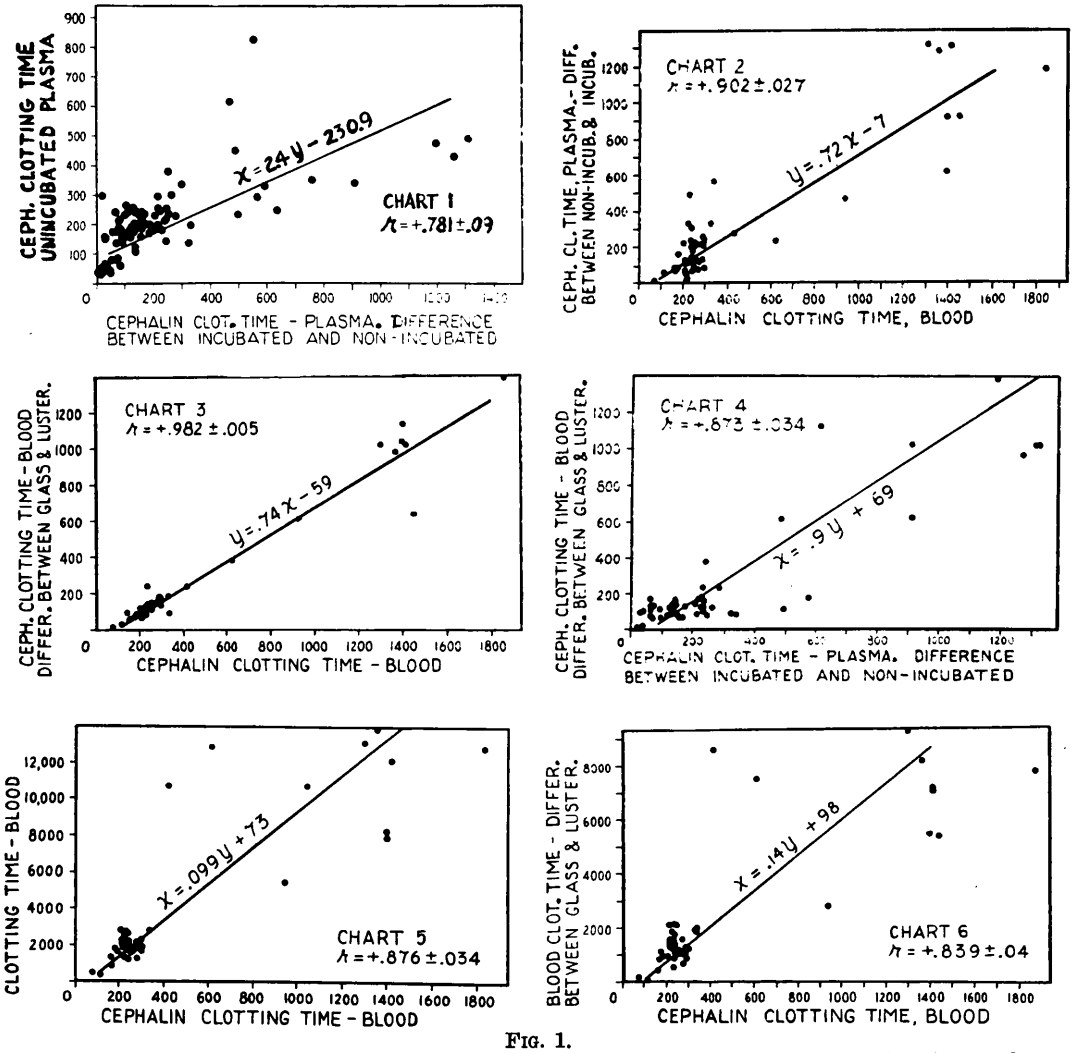

FIG. 1.

Correlations between the rate of coagulation of human blood and plasma in glass and lusteroid tubes, with and without incubation with cephalin. Unless otherwise specified, the clotting times (expressed in seconds) refer to determinations in lusteroid tubes. No determinations included in the above group if the prothrombin was lower than 70% of the normal, or if there was an excess of antithrombin (heparinized blood).

the plasma (Table II) and may in part result from artifacts introduced during the separation, collection, standing, and recalcification of the plasma. An indication, however, that some of the anticephalin effect is usually lost during these steps is obtained by comparing the cephalin clotting times of blood in the 2 tubes with that of the respective plasmas (unincubated) (Tables I and II). While in normal subjects the times on plasma and blood in the same tube are almost alike, in fresh hemophilic blood the cephalin clotting time in the two tubes may be more than twice as long as that of its unincubated plasma. This supplies an additional reason for testing this function in fresh blood, without anticoagulants.

The superimposition of hypoprothrombinemia or heparinemia would naturally exaggerate the anticephalin effect. The need of a prothrombin determination is therefore apparent for the proper estimation of the significance of a prolonged cephalin clotting time of venous blood. This determination may be con-

TABLE II.
The Coagulation Time in Glass and Lusteroid Tubes of Venous Blood with and without Additions of Cephalin, and of Plasma before and after Incubation with Cephalin. Human Subjects with Various Hemorrhagic Disorders.

	Subject											
	1 Post-hemorrhagic, with thrombopenia		2 Post-hemorrhagic, with thrombocytosis		3 Thrombopenic (nonhemorrh.)		4 Hemophilic		5 Hypoprothrombinemic (dicoum.)		6 Heparinized†	
Determination*	Glass	Lust.	Glass	Lust.	Glass	Lust.	Glass	Lust.	Glass	Lust.	Glass	Lust.
Clot. time, blood (sec)	360	480	480	420	540	1440	5100	12900	690	2760	1860	2940
Ceph. clot. time, blood (sec)	75	81	86	112	99	201	830	1449	204	579	220	480
Ceph. clot. time, plasma 0′ incub. (sec)	74	90	71	96	87	192	238	618	92	235	203	792
20′ incub. (sec)	80	109	76	64	100	329	345	2296	157	860	532	>3600
Blood platelets (thous./mm³)	20		510		18		430		180		154	
Plasma prothr. (%)												
1-stage meth.	90		100		90		100		22		95	
2-stage meth.	72		70		108		110		20		90	

* For methods see footnotes under Table I.
† 40,000 units Lederle Heparin intravenously over a period of 48 hours. Man, wt 65 kg.

veniently done at the time the blood is collected by one of the standard bedside methods,[2] or in the plasma by the two-stage method.[5] Naturally occurring increases in the antithrombin content of the blood are seldom encountered, and require no especial technical provision in clinical studies.

Summary. The rate of coagulation of whole blood in paraffin coated or lusteroid tubes after addition of a standard suspension of cephalin, gives a rapid and reliable estimation of the anticephalin activity of the corresponding plasma. The extent of the difference between the rate of coagulation of blood in glass and lusteroid tubes supplies an additional estimate of this activity. The response to the clot accelerating action of cephalin does not seem to be significantly affected by the platelet content of the blood; the presence of hypoprothrombinemia may, however, exaggerate the delay in the response.

[5] Smith, H. P., Warner, E. D., and Brinkhous, K. M., *J. Exp. Med.*, 1937, **66**, 801.

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Effect of Penicillin and Patulin on Fowl Pox.*

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The effects of penicillin and patulin upon the infection of fowl pox, contagious epithelioma, of the chorioallantoic membrane of the developing chick embryo have been determined.

Procedure. Forty-five eggs which had been incubated for 11 days were prepared for inoculation by the technic described by Good-

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