due to the fact that, in the work presented here, the blood was taken directly from the limb, the saphenous vein of the cat, while in the previous work, the renal vein was the source of the blood samples. The increase of choline-esterase found under those circumstances may be due to some other factor in some other part of the body rather than to a direct output of choline-esterase by the stimulated muscles.

I wish to acknowledge my indebtedness to Dr. W. O. Fenn and Dr. J. C. Sabine for their assistance in both technical problems and interpretation of results.

13194

Action of Nicotinic Acid on Coagulation of the Blood.

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Calder and Kerby[1] recently reported that the prolonged coagulation time of heparinized blood could be significantly shortened *in vitro* by the addition of 1% nicotinic acid. They stated " . . . . that nicotinic acid does not duplicate the action of, nor can it be substituted for, any of the known factors involved in blood clotting," and furthermore suggested that its effect might be due to chemical neutralization of antithrombin.

The following experiments were performed in order to investigate the mechanism of action of nicotinic acid on the coagulation of the blood.

Methods. The coagulative potency of 1% nicotinic acid in 0.85% NaCl; 1% nicotinic acid amide in 0.85% NaCl; Nicamin,* representing 1% nicotinic acid; 0.001% protamine;** thromboplastin solution;[2] 0.85% NaCl; and distilled water was tested with the blood of 4 healthy human adults in whom the bleeding time, coagu-

† Assisted by grants from Mr. Frank Kennedy and the Pacific Institute of Tropical Medicine.

1 Calder, R. M., and Kerby, G. P., *Am. J. Med. Sci.*, 1940, **200**, 590.

* Monoethynolamine nicotinate, Abbott. Dilutions of the commercial product representing 25 mg of nicotinic acid per cc were made with 0.85% NaCl.

** Kindly supplied by Eli Lilly & Co.

2 Quick, A. J., *J.A.M.A.*, 1938, **110**, 1658.

lation time, blood clot retraction, prothrombin concentration, and platelet count was normal. All tests were done in a water bath at 37°C and were timed with a stopwatch.

Plasma Experiments: These tests were performed in triplicate by methods similar to those previously reported.[3] Glass test tubes were used (10 x 75 mm), 9 cc of blood was obtained by venipuncture, immediately mixed with 1 cc of 1.34% sodium oxalate solution and centrifugalized at 2000 r.p.m. for 30 minutes. 0.1 cc of a 0.85% NaCl solution containing 2 units of heparin‡ was added to each cc of the supernatant plasma. 0.1 cc heparinized plasma was mixed with 0.1 cc of each of the substances to be tested. 0.1 cc of a 0.27% $CaCl_2$ solution was added to each mixture and the coagulation time noted. Control tests with 0.85% NaCl and thromboplastin solutions were done with unaltered plasma and whole blood.

Whole Blood Experiments: These tests were performed in duplicate. Glass test tubes (13 x 100 mm) were used, 0.2 cc of a 0.85% NaCl solution containing 0.75 units of heparin‡ per cc was mixed with 0.9 cc of each of the test substances. 1.5 cc of whole blood obtained by venipuncture was immediately added to each tube and the coagulation time noted.

Hemolytic Tests. 1.5 cc of a 2% suspension of washed sheep cells was added to 1.5 cc of each test substance and observed for hemolysis, immediately and at intervals up to 1½ hours.

Results. The results of the plasma experiments are given in Table I. The coagulation time remained prolonged beyond 1800 seconds in all samples to which nicotinic acid, nicotinic acid amide, Nicamin, 0.85% NaCl, or distilled water had been added. The average coagulation time was shortened to 238 seconds in the samples to which protamine was added, and to 36 seconds in the samples to which thromboplastin was added. In the control samples the average coagulation times were 173 and 15½ seconds. In a separate series of experiments it was determined that 0.001% protamine alone is slightly anti-coagulant when tested with plasma.

It is apparent that nicotinic acid, nicotinic acid amide and Nicamin are ineffectual when tested with heparinized plasma.

The results of the whole blood experiments are given in Table II. The coagulation time remained prolonged beyond 3600 seconds in the samples to which nicotinic acid amide, Nicamin, or 0.85% NaCl were added. It was shortened to an average of 1037 seconds in the

[3] Aggeler, P. M., and Lucia, S. P., *Am. J. Med. Sci.*, 1940, **199**, 181.

‡ Connaught Laboratories—1000 units per cc.

TABLE I.
Effect of Various Test Substances on Coagulation of Human Plasma.

	Substance	Coagulation Time in Seconds				
		Subj. A	Subj. B	Subj. C	Subj. D	Average
Heparinized Plasma +	0.85% NaCl	>1800	>1800	>1800	>1800	>1800
	Distilled water	>1800	>1800	>1800	>1800	>1800
	Nicotinic acid	>1800	>1800	>1800	>1800	>1800
	" " amide	>1800	>1800	>1800	>1800	>1800
	Nicamin	>1800	>1800	>1800	>1800	>1800
	Protamine	238	236	238	240	238
	Thromboplastin	43	32	34	35	36
Unaltered Plasma +	0.85% NaCl	215	163	166	150	173
	Thromboplastin	15	15	16	16	15½

samples to which nicotinic acid was added, to 1144 seconds in distilled water, to 349 seconds in protamine, and to 29 seconds in the samples to which thromboplastin was added. In the control samples the average coagulation times were 243 and 10 seconds.

It is apparent that the limited effect of nicotinic acid is similar to that of distilled water and that the two compounds of nicotinic acid are without significant effect.

In order to further elucidate the problem, it seemed advisable to investigate the hemolytic effect of the test substances. The results of the tests are given in Table III. Distilled water induced immediate and complete hemolysis. Following an initial delay of several minutes, the nicotinic acid appeared to promote complete hemolysis with the production of a brownish tinged fluid, characteristic of acid hematin. Within 1½ hours a flocculant mass of laked red blood cells and cellular debris had settled to the bottom of the tube.

From these experiments it appears that nicotinic acid induces coagulation of heparinized blood by damaging the formed elements contained therein. Such action would release thromboplastin and

TABLE II.
Effect of Various Test Substances on Coagulation of Human Blood.

	Substance	Coagulation Time in Seconds				
		Subj. A	Subj. B	Subj. C	Subj. D	Average
Heparinized Whole Blood +	0.85% NaCl	>3600	>3600	>3600	>3600	>3600
	Distilled water	1230	885	1560	900	1144
	Nicotinic acid	967	1080	1100	990	1037
	" " amide	>3600	>3600	>3600	>3600	>3600
	Nicamin	>3600	>3600	>3600	>3600	>3600
	Protamine	330	347	345	375	349
	Thromboplastin	40	38	18	20	29
Whole Blood +	0.85% NaCl	200	247	285	240	243
	Thromboplastin	10	9	10	11	10

TABLE III.
Comparative Hemolytic Effect of Test Substances on Sheep Erythrocytes.

Substance	Degree of Hemolysis
0.85% NaCl	0
Distilled water	++++
Nicotinic acid	++++
" " amide	+
Nicamin	+
Protamine	0
Thromboplastin	0
Heparin solution	0

thus accelerate the conversion of prothrombin to thrombin, thereby overcoming the anticoagulant action of heparin.[4]

Summary. Nicotinic acid has no coagulant effect when tested *in vitro* with heparinized recalcified plasma. Its coagulant action is comparable to that of distilled water when tested *in vitro* with heparinized whole blood. Nicotinic acid is an active hemolytic agent and its coagulant effect is apparently due to the release of thromboplastin from the disrupted elements of the blood. Compounds of nicotinic acid which do not produce significant hemolysis have no appreciable coagulative potency.

13195 P

Effect of Milk on Gizzard Erosion and Cholic Acid in the Chick.

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The preventive and curative effect of cholic acid on dietary gizzard erosion of chicks has been reported.[1, 2] The effect is similar in the case of gizzard erosions produced by the use of either a basal diet or a practical rearing diet to which cincophen has been added.[2] We desire to report at this time evidence for the existence in cow's milk of a labile substance which acts like cholic acid.

Dried milk products were fed by mixing in the diets; liquid milk products were given to the chicks in place of the drinking water.

[4] Quick, A. J., *Am. J. Physiol.*, 1936, **115**, 317.

[1] Almquist, H. J., and Mecchi, E., *J. Biol. Chem.*, 1938, **126**, 407.

[2] Almquist, H. J., and Mecchi, E., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 168.