its effect on various enzymes studied, for example, trypsin,[4, 11] leucoprotease,[12] lipase, etc. Blanc and Pozerski[6] found decreased power of gelatin liquefaction of *Cl. histolyticum* filtrates in the presence of raw serum albumin. Other enzymes have been studied more extensively in this respect.[13] Furthermore, the result of repeated injection of such materials into animals is to increase the antienzymatic effect of the serum[14, 15] and to produce antibodies.[16] The exact nature of the substance involved in the antienzymatic mechanism it not yet completely understood. It seems reasonable to assume from our experience and that of others with *in vitro* experiments, that such fibrinolytic filtrates would not be effective *in vivo*.

Summary. 1. Filtrates of *Cl. histolyticum* grown on various protein-rich media possess a high fibrinolytic potency, whether sulphydryl activator be present or not. 2. This fibrinolytic potency is greatly depressed in the presence of dog serum. 3. It is probable that the use of filtrates of *Cl. histolyticum* as a fibrinolytic agent in dissolving fibrin deposits *in vivo* would not be feasible due to the antienzymatic properties of blood serum.

I am grateful to Dr. L. N. Katz, to Dr. K. Howell, and to Dr. I. Kaplan for helpful suggestions and advice.

10301

Heparin and the Blood Coagulation Mechanism.*

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The efficacy of heparin as an anticoagulant was first observed by Howell and Holt.[1] Later Howell[2] demonstrated the presence of heparin in the blood and described a method for its purification.

[11] Banting, F. G., and Gairns, S., *Am. J. Physiol.*, 1930, **94**, 241.

[12] Opie, E. L., *J. Exp. Med.*, 1906, **8**, 536.

[13] Opie, E. L., *Physiol. Rev.*, 1922, **2**, 552.

[14] Achalme, P., *Ann. de l'Inst. Pasteur*, 1901, **15**, 737.

[15] Bergmann, von, und Bamberg, *Berl. klin. Wochenschr.*, 1908, **45**, 1396.

[16] Connell, H. C., *Bull. Hendry-Connell Research Foundation*, 1938, **3**, 3.

* Assisted by a grant from the Christine Breon Fund.

[1] Howell, W. H., and Holt, E., *Am. J. Physiol.*, 1918, **47**, 328.

[2] Howell, W. H., *Am. J. Physiol.*, 1924, **71**, 553.

Quick[3, 4] demonstrated that, although heparin itself is not an antithrombin, it does "appear to react with a constituent present in plasma to form a true antithrombin." He also presented data to show that thromboplastin does not neutralize heparin. Removal of the electrolytes (and perhaps of a "diffusible organic substance"[5]) from plasma renders heparin inactive. Eagle[6] stated that "it is debatable whether heparin is of *physiologic*† significance in preventing intravascular coagulation," but concluded in his summary that "heparin apparently prevents coagulation both by retarding the formation of thrombin and by acting as an antithrombin." Charles and his coworkers[7, 8] prepared a potent product of heparin which could be administered intravenously. Murray and Best and their coworkers[9, 10] used this material (70 units per mg) on animals and human subjects and demonstrated that it greatly reduced or prevented the tendency to vascular occlusion in traumatized veins. Crafoord[11] used heparin (prepared by Jorpes) post-operatively in human subjects and noted that it produced prolongation of the coagulation time of the blood without any toxic after-effects.

Our experiments consisted of determinations of the effects of heparin‡ on the coagulation time of whole blood and plasma *in vitro* and *in vivo* in normal and in diseased subjects.

All experiments were performed in a water-bath maintained at a temperature of 37°C. The Lee and White method for the coagulation time of whole blood was used. The methods of Quick, with slight modifications, were employed for the coagulation time of recalcified plasma, and of recalcified plasma to which an addition of thromboplastin was made.[12, 13, 14] A 5% emulsion of fresh rabbit

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3 Quick, A. J., *Am. J. Physiol.*, 1936, **115**, 317.

4 Quick, A. J., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 391.

5 Larson, C. E., and Greenberg, D. M., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 305.

6 Eagle, H., *Medicine*, 1937, **16**, 95.

† Author's italics.

7 Charles, A. F., and Scott, D. A., *J. Biol. Chem.*, 1933, **102**, 425, 437.

8 Charles, A. F., and Scott, D. A., *Trans. R. S. Can.*, 1934, **28**, Sec. V, 55.

9 Murray, D. W. G., Jaques, L. B., Perrett, T. S., and Best, C. H., *Can. Med. Assn. J.*, 1936, **35**, 621.

10 Best, C. H., Cowan, C., and MacLean, D. L., *Science*, 1937, **85**, 338.

11 Crafoord, C., *Acta Chir. Scand.*, 1937, **79**, 407.

‡ Heparin obtained from the Connaught Laboratories. A sterile solution for intravenous use, 1000 units per cc. One unit of this product will keep 1 cc of cat blood free of clots for 24 hours in cold-storage.

12 Quick, A. J., Stanley-Brown, M., and Bancroft, F. W., *Am. J. Med. Sci.*, 1935, **190**, 501.

13 Quick, A. J., *Am. J. Physiol.*, 1936, **114**, 282.

14 Aggeler, P. M., and Lucia, S. P., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 11.

brain provided the source of thromboplastin. The results of the experiments undertaken are tabulated; in each instance the figures shown were characteristic of all experiments of the series.

EXPERIMENT I.

Effect of Varying Concentrations of Heparin on the Coagulation Time of Normal Whole Blood and Plasma *in vitro*. (Results in one of 5 experiments.)

Concentration of heparin-units per cc	Coagulation Time		
	Whole blood	Recalcified plasma, saline control	Recalcified plasma with added thromboplastin
1.5	no clot in 360′	75′0″	720″
1.0	46′	10′0″	42″
0.5	35′	8′15″	35″
0.25	25′	5′45″	27″
0.1	9′	3′10″	22″
0.05	5′	2′45″	22″
Control	5′	2′10″	22″

EXPERIMENT II.

Effect on Coagulation Time of Whole Blood and Plasma of a Single 3 cc Dose (3000 Units) of Heparin Administered Intravenously to a Normal Subject. (Results in one of 5 experiments.)

Interval	Coagulation Time		
	Whole blood	Recalcified plasma, saline control	Recalcified plasma with added thromboplastin
Before heparin (control)	5′	2′10″	22″
5 min. after heparin	53′	5′45″	39″
1 hr ″ ″	25′	4′10″	29″
2 ″ ″ ″	12′	2′15″	24″
3 ″ ″ ″	6′	2′10″	22″
4 ″ ″ ″	5′	2′12″	22″

EXPERIMENT III.

Effect on Coagulation Time of Whole Blood and Plasma of a Single 3 cc Dose (3000 Units) of Heparin Administered Intravenously to a Hemophiliac and a Patient with Icterus (Index 125).

Interval	Coagulation Time			
	Whole blood		Recalcified plasma with added thromboplastin	
	Hemophilia	Icterus	Hemophilia	Icterus
Before heparin (control)	45′	5′	21″	29″
5 min after heparin	240′	42′	39″	45″
1 hr ″ ″	135′	23′	26″	34″
2 ″ ″ ″	90′	12′	21″	29″
3 ″ ″ ″	48′	6′	20″	28″
4 ″ ″ ″	44′	6′	21″	29″
Normal patient control	6′	5′	22″	22″

Comparing Experiments II and III reveals that the coagulation time of the whole blood of the hemophiliac differs markedly from

the normal, while that of the recalcified plasma remains similar. In icterus, it is the coagulation time of the recalcified plasma which differs from the normal.

EXPERIMENT IV.

Effect on Coagulation Time of Whole Blood of Single Graded Doses of Heparin Administered Intravenously to Normal Subjects. (Results in one of 5 experiments.)

Interval	Coagulation time of whole blood after 1 cc	2 cc	3 cc	4 cc	6 cc
Before heparin (control)	5′	4′30″	5′	7′30″	5′
5 min after heparin	17′	27′	53′	90′	100′
30 ″ ″ ″	13′	22′			
1 hr ″ ″	11′	15′	25′	53′	60′
1½ ″ ″ ″	6′30″	11′			
2 ″ ″ ″	5′	8′	12′	28′	37′
3 ″ ″ ″		5′	6′	15′	16′
4 ″ ″ ″			5′	8′	9′
5 ″ ″ ″				7′	5′

A relatively large dose of heparin given intramuscularly is practically without effect. A 5 cc intramuscular dose given simultaneously with a 3 cc intravenous dose did not prolong the coagulation time of the whole blood significantly insofar as intensity or duration of the effect is concerned. A continuous intravenous drip of salt solution delivering 1000 units of heparin per hour was without effect. If an initial intravenous dose of 3 cc of heparin is given and followed by a continuous intravenous dose of 1 cc (1000 units) per hour, the coagulation time of the whole blood may be kept at 30 minutes for periods of 8 hours or more.

EXPERIMENT V.

Effect on Coagulation Time of Whole Blood and Plasma of Repeated Single Doses of Heparin. (One of 4 experiments.)

Interval	Coagulation Time: Whole blood	Recalcified plasma, saline control	Recalcified plasma with added thromboplastin
Before heparin (control)	7′	2′15″	22″
3 cc heparin intravenously			
5 min. after heparin	48′	4′30″	33″
1 hr ″ ″	28′	2′33″	28″
2 ″ ″ ″	14′	2′30″	26″
2 cc heparin intravenously			
5 min after heparin	50′	3′25″	30″
1 hr ″ ″	26′	2′45″	27″
2 ″ ″ ″	15′	2′40″	25″
2 cc heparin intravenously			
5 min after heparin	54′	3′45″	33″
1 hr ″ ″	31′	3′02″	27″
2 ″ ″ ″	20′	2′55″	25″
3 ″ ″ ″	10′	2′30″	23″
4 ″ ″ ″	8′	2′20″	22″

In 2 persons suffering from coronary artery thrombosis, 3 cc doses of heparin repeated at 3 hourly intervals were given for periods of 5 and 7 days. The coagulation time was kept prolonged throughout this period.

During the course of these experiments some evidence was obtained to indicate that the heparin injected was probably destroyed in the body and not voided in the urine. The bleeding time (Duke method), capillary fragility (Daldorf method) and clot retraction after heparin are normal. Leucocytes and bone marrow pulp left in contact with heparin for periods greater than one hour tend to agglutinate and later disintegrate. There is a tendency for hematomata to form at the site of venipuncture after heparin unless the wound is carefully tamponed.

Summary. Heparin added to whole blood *in vitro* or administered by vein prolonged the coagulation time of whole blood and plasma. The coagulation time was prolonged and maintained by a single large dose of heparin followed by a smaller continuous dose. The mechanism appeared to be independent of the presence and type of disease. Heparin produces agglutination and disintegration of leucocytes and bone marrow pulp *in vitro*.

10302

Serological Studies on Mastitis.

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One of the great difficulties encountered in studies on the streptococcal type of mastitis is the diagnosis of the chronic form of the disease. Both the chemical and bacteriological methods have their proponents, but as yet, no one test or series of tests can be considered as a final diagnostic measure. For this reason it would seem that any valuable means of diagnosis must bring in methods differing greatly from those already recommended.

Despite the fine work done on certain animal diseases by applying serological methods this particular field seems to have been practically neglected by those interested in mastitis. This appears particularly strange in view of the fact that as early as 1904 Stahli[1] was able to

[1] Stahli, A. T., *Arch. f. wessensch. u. prakt. Tierheilk.*, 1904, **30**, 374.