and experimental dogs were, respectively, 69 and 81 of the chloride; 24 and 30 of the sulfate; and 34 and 44 of the fluid.

In both control and experimental animals the sulfates showed a definite increase in concentration at the end of the experimental period, the chlorides a definite impoverishment. However, the differences between the control and the experimntal animals were not statistically significant.

16058

Influence of Iodinated Trypsin on Blood Coagulation.*

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It has been known for some time that the addition of trypsin accelerates the coagulation of normal blood[1-4] and the blood of hemophilic individuals.[5,6] Intravenously administered trypsin is, however, quite toxic as pointed out by Eagle and Harris.[4] The similarity between the symptomatology resulting from the injection of the enzyme and anaphylactic reactions was observed by Rocha e Silva[7] and these observations have been extended by Dragstedt and his associates.[8-12] Shock resulting from the injection of a relatively small amount of trypsin is indicated by a marked fall in blood pressure.

Tagnon[13] slowly injected trypsin intravenously in hemophilic patients and observed a significant shortening of the coagulation time; however, he has advised extreme caution since the dose most effective on clotting time is very near to that which is quite toxic.

In an earlier report[14] the author pointed out that the iodination of trypsin serves to decrease greatly its hypotensive effect while its proteolytic activity is decreased only about one tenth as much. Relatively large amounts of the iodinated enzyme do not cause the profound fall in blood pressure observed with the same amount of untreated trypsin even with rapid injection.

The present report deals with the influence of iodinated trypsin on the rate of blood coagulation *in vitro*.

Methods. Freshly drawn dog blood was mixed with 3.3 mg of sodium citrate per ml of blood. The plasma was separated after centrifuging for 2 hours at 3200 r.p.m. With short periods of centrifugation the clotting

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TABLE I.
Acceleration of Coagulation by Iodinated and Untreated Trypsin. Each tube contained 0.5 ml of plasma and 0.1 ml of 1% $CaCl_2 \cdot 2H_2O$ plus other additions as indicated.

No.	NaCl 0.9% ml	Non-iodinated trypsin 0.1% ml	Iodinated trypsin: 1.6 × 10⁻³ m Eq iodine per mg trypsin	Iodinated trypsin: 1.8 × 10⁻³ m Eq iodine per mg trypsin	Iodinated trypsin: 2.0 × 10⁻³ m Eq iodine per mg trypsin	Avg coagulation time, sec	% of normal coagulation time
1	0.1	0	0	0	0	755	100.0
	0	0.1	0	0	0	68	9.0
	0	0	0.1 ml of 0.3%	0	0	75	9.9
2	0.1	0	0	0	0	221	100.0
	0	0.1	0	0	0	37	16.7
	0	0	0.1 ml of 0.1%	0	0	47	21.2
	0	0	0.1 ml of 0.3%	0	0	35	15.8
3	0.1	0	0	0	0	338	100.0
	0	0.1	0	0	0	47	13.9
	0	0	0	0.1 ml of 0.3%	0	50	14.8
4	0.1	0	0	0	0	475	100.0
	0	0.1	0	0	0	54	11.4
	0	0	0	0.1 ml of 0.3%	0	65	13.7
5	0.1	0	0	0	0	358	100.0
	0	0.1	0	0	0	54	15.1
	0	0	0	0	0.1 ml of 0.1%	95	26.5
6	0.1	0	0	0	0	430	100.0
	0	0.1	0	0	0	65	15.1
	0	0	0	0	0.1 ml of 0.1%	116	27.0
	0	0	0	0	0.1 ml of 0.3%	76	17.7
7	0.1	0	0	0	0	130	100.0
	0	0.1	0	0	0	41	31.5
	0	0	0	0	0.1 ml of 0.3%	44	34.0
	0	0	0	0	0.1 ml of 0.5%	40	30.9
8	0.1	0	0	0	0	127	100.0
	0	0.1	0	0	0	41	32.2
	0	0	0	0	0.1 ml of 0.1%	48	37.8
	0	0	0	0	0.1 ml of 0.3%	44	34.6
	0	0	0	0	0.1 ml of 0.5%	42	33.0

time was considerably less and the accelerating influence of untreated or iodinated trypsin was less apparent. Plasma and the diluent or the enzyme were placed in tubes having inside measurements of 5 x 55 mm and allowed to come to 38°C in a water bath. Calcium chloride was then added, a stopper quickly inserted and the tubes were slowly and uniformly rocked back and forth under the surface of the 38° bath until the air bubble in the tube became stationary and a clot was observed. With these small tubes it was found desirable to begin timing the coagulation when the tubes were first inverted with the calcium chloride present.

The crude trypsin was iodinated under conditions described earlier.[14]

Results. The data of Table I indicate that iodinated trypsin, as well as the untreated enzyme, considerably hastens the coagulation of recalcified plasma. The difference in the degree of acceleration by the untreated and by the iodinated enzyme is similar to the difference in their proteolytic activities described earlier.[14] Approximately the same degree of acceleration by iodinated trypsin can be accomplished by employing 3 to 5 times as much material. However, like the rate of proteolysis, acceleration of coagulation is not directly proportional to the concentration of the enzyme and appreciable acceleration results when iodinated trypsin is used

in amounts equal to the optimum amount of the untreated material.

Further study of the numerous factors to be considered in the use of iodinated trypsin *in vivo* is in progress.

Summary. The coagulation of recalcified plasma, *in vitro,* is hastened considerably by trypsin which has been iodinated sufficiently to greatly decrease its hypotensive effect.

16059

Depth of Penetration of Nebulized Substances in the Respiratory Tree.*

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(Introduced by E. Ogden.)

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To effect a local concentration of penicillin in the pulmonary system, some physicians have been administering nebulized penicillin in the inspired air.

Administering penicillin mist by the usual method of inserting a spray into the oral pharynx uses two routes of administration simultaneously. The mist deposited on the pharyngeal mucosa is absorbed, diluted by the blood stream and distributed throughout the body; also some of the mist may follow the column of inspired air down the respiratory passages to effect a local surface action. Intramuscular injection of penicillin produces higher blood concentrations than is possible by mucosal absorption of the relatively small dosage used in nebulizers. Thus, the only advantage of nebulization is a possible local action on the surfaces of the respiratory passages.

The purpose of this study was to determine the magnitude of concentration and the depth of penetration down the respiratory passages, of a nebulized substance administered with inspired air.

Liquids, such as Lipiodol, pass rapidly to the terminal bronchioles and alveoli. It is known from unpublished war research that aerosol clouds in which the mass median diameter is carefully controlled will penetrate in small concentrations to the alveoli. Dusts, such as silica, over long periods of exposure may penetrate deeply. This study is not concerned with such phenomena, but only with conditions similar to those of aerosol therapeusis, *i.e.,* mixture, with commercial nebulizers, of mist with inspired air in the oral pharynx, and durations of exposure of 15-60 minutes of eupneic breathing.

Nebulized particles in inspired air tend to be deposited against the walls of the respiratory system by settlement due to gravity and by changes in the direction of air flow. The particles tend to be retained in the system by impingement on moist and adhesive surfaces. The deposition due to changes in direction of air flow increases with the velocity of air flow and turbulence, whereas settlement, due to gravity, is only effective where the velocity is low.

Hatch[1] states that maximum air velocities occur in the nasal passages. The mean velocity of the air column in the bronchioles is reduced to 1/1000, and at the entrance of the alveoli is further reduced to 1/100,000 of nasal velocity. Thus the greatest deposition of nebulized particles should be expected in the upper respiratory tree. The air velocity

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