virus. In order to produce this effect in mice, virus of relatively high infectivity was used. Following criss-cross passages between tissue cultures and mice the infectivity of this strain was increased almost 10-fold when titrated in tissue cultures.

The dissociation between ID_{50} inducing paralysis or death when given peripherally or centrally is striking. From this it might seem that only small amounts of virus injected intravenously will reach the CNS. Poliomyelitis virus has been demonstrated in other than nervous tissues following infection in man as well as in animals (blood, lymphnodes and spleen). Though it is questionable whether poliomyelitis virus multiplies in non-neural tissues *in vivo* the presence of virus in these organs following intravenous inoculation may result in a rapidly developing immunity which might interfere with the occurrence of the paralytic disease when virus doses are given that result in an incubation period of more than 10 days.

Summary. Poliomyelitis virus Type 2 has been successfully propagated in mice following intravenous inoculation. 10^7 ID_{50} tissue culture doses were required to produce 50% response (paralysis or death) after intravenous inoculation in mice. The intravenously inoculated virus disappeared from the serum after 24 hours but was recoverable from spinal cord, brain and feces.

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Influence of Bean Trypsin Inhibitors on Thromboplastic Properties of Trypsin and Hypochlorite Treated Trypsin.* (21144)

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That trypsin is capable of accelerating the coagulation of blood is well known(1,2) and it has been repeatedly observed that bean trypsin inhibitors retard coagulation(3-6). The present report deals mainly with the thromboplastic properties of trypsin or hypochloric treated trypsin combined with bean trypsin inhibitors. The differentiation of the inhibitors used is briefly considered.

Methods. Coagulation time was determined as described earlier(7). Proteolytic activity was estimated according to the method of Kunitz(8). The inhibition of proteolytic activity was measured by determining the amount of inhibited enzyme required to accomplish the same degree of digestion of a given solution of casein as did a known amount of the untreated enzyme. Inhibitor solutions were added to enzyme solutions at pH 7.4 ten minutes before use. The globulin soy bean trypsin inhibitor, GSTI, was prepared according to the method of Kunitz(9) who has described the properties of the crystalline material(8). The navy bean trypsin inhibitor, NTI, was prepared as follows: 800 g of finely ground navy beans were extracted with 4 liters of water at 50°C for 18 hours. After centrifuging, trichloroacetic acid was added to the supernate to 2.5% concentration. The preparation was heated to 80 to 85°C for 5 minutes and centrifuged while hot. The

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supernate was adjusted to pH 6.5 with NaOH after cooling to 25°C. Sufficient 95% ethyl alcohol was added to bring the concentration to 60%; the resulting precipitate was discarded after centrifuging, and the alcohol content was increased to 75%. After standing overnight at room temperature most of the supernatant solution was removed by siphoning; the precipitate was then collected by centrifuging and dried in a vacuum desiccator. Yields varied from 0.3 to 0.5% of the bean meal. Preparations of greatest activity were obtained from newly harvested beans. The protease soy bean trypsin inhibitor can be prepared by the above procedure except for the adjustment to pH 9 prior to adding alcohol. Hypochlorite-treated trypsin was prepared as described earlier(7). Rabbits were used because of their marked susceptibility to pulmonary and cardiac embolism with the intravenous injection of trypsin. The term trypsin refers to crystalline trypsin unless otherwise indicated and Cl-trypsin refers to hypochlorite-treated trypsin. Throughout this investigation observations were made with both GSTI and NTI with comparable results. Data are given for the former because of its crystalline nature although the properties of the latter may offer advantages for *in vivo* administration.

Results. The intravenous administration of the bean trypsin inhibitors with trypsin causes considerably less fatal embolism in rabbits than does the enzyme alone. For example, the injection of 5 mg of trypsin/kg was fatal in all of 10 rabbits as was 10 mg/kg in an equal number. However, 10 daily injections of the enzyme in 10 mg/kg doses with an equal amount of GSTI was tolerated by 10 animals without response. The emboli observed in the fatal cases were large and unmistakable, frequently filling the pulmonary arteries and right auricle. They were observed immediately after cessation of respiration which occurred within a few minutes after injection. Death was preceded by marked symptoms of anoxemia.

TABLE I. Thromboplastic and Proteolytic Activities of Inhibitor-Enzyme Complexes *In Vitro*.

Enzyme: inhib. ratio	% of control coagulation time*		Proteolytic activity†	
	Trypsin + GSTI	Cl-trypsin + GSTI	Trypsin + GSTI	Cl-trypsin + GSTI
1:3	142	161	0	0
1:2	70	121	0	0
1:1	21	40	2	0
1:0.5	3	25	15	5

* In each case 0.6 mg protease used/ml of plasma.

† % of the uninhibited activity of the enzyme as estimated by casein digestion.

TABLE II. Thromboplastic Effect of Inhibitor-Enzyme Complexes Injected by Jugular Vein.

Enzyme, mg/kg	GSTI, mg/kg	% of control coagulation time after inj. 5 min.	60 min.	180 min.
		Trypsin		
2*	0	26	42	103
5	5	28	47	51
5	10	45	57	60
		Cl-trypsin		
5	0	40	47	71
5	5	89	76	48
5	10	100	102	109

* 5 mg/kg caused fatal embolism.

Although an equal weight of GSTI administered with trypsin reduced embolism the complex retains marked clot accelerating properties. This was observed *in vitro* (Table I) and after intravenous administration (Table II). Appreciable clot acceleration occurs even when the enzyme:inhibitor complex shows little or no proteolytic activity; and the injection of the uninhibited enzyme in amounts sufficient to provide proteolytic capacity equivalent to that of the 1:1 complex did not alter clotting time. On the other hand, relative protection from embolism is observed even with an enzyme:inhibitor complex showing considerable proteolytic activity. Commercial trypsin differs from the crystalline enzyme in that approximately half of its original proteolytic activity is retained in the presence of 2 or 2.5 times as much GSTI or NTI. Yet the inhibitors decidedly diminish embolism (Table III) in spite of this residual proteolytic activity. Again, markedly decreased clotting times were observed.

Therefore, it would appear that the enzyme:inhibitor complex does not influence the blood clotting mechanism through its initial

TABLE III. Influence of Bean Trypsin Inhibitors on Trypsin Embolism in Rabbits.

No. of animals	Material inj. by ear vein	No. of fatalities	No. with gross emboli	Amt of trypsin inj., mg/kg*		
				Avg total	Max	Min
44	Trypsin (commercial)	40	38	8	19	2
20	" GSTI (1:2)	2	1	20	30	10
20	" NTI (1:2.5)	3	1	22	27	8
20	Cl-commercial trypsin	14	14	18	31.5	3
23	*Idem*, NTI (1:2.5)	5	5	14.5	22	3

* Injected at rate of 1 mg enzyme/kg/15 min.

free proteolytic effect; although plasma activated with acetone(7) did show a significant increase in proteolytic activity after the complex was added *in vitro* or injected. This occurred without change in the natural inhibitor level of plasma prior to acetone treatment. Continued study of the problem may provide further information relative to the controversy over the participation of proteolytic factors in the natural blood clotting process.

The thromboplastic properties of the enzyme:inhibitor complex in which hypochlorite-treated trypsin is substituted for the untreated enzyme are similar to those described above (Tables I, II, III). The properties of uninhibited Cl-trypsin have been described(7). Although the bean trypsin inhibitors do not diminish the hypotensive effect of trypsin(10) such diminution in marked degree results from treating the enzyme with hypochlorite(7). Therefore, suitable amounts of the crystalline enzyme so treated and combined with the GSTI has low hypotensive and proteolytic activities with an appreciable thromboplastic effect. Optimal quantities for the thromboplastic effect of the 1:1 complex *in vitro* are given in Table IV. The duration of decreased coagulation time after injecting

TABLE IV. Thromboplastic Activity of Varying Amounts of Cl-crystalline Trypsin in Presence of Equal Amounts of GSTI and without Inhibitor *In Vitro*.

mg enzyme/ml plasma	% of control coagulation time	
	With GSTI	Uninhibited
.015	51	8
.044	48	7
.291	43	6
.582	45	6
1.165	75	4
1.75	78	5
2.33	93	5
2.91	105	Incoagulable

trypsin or Cl-trypsin with an equal amount of inhibitor is generally more prolonged than with the uninhibited enzyme administered in sufficient amounts to produce about the same initial clot acceleration.

In view of the differing behavior and properties of the GSTI and the proteose inhibitors of soy and navy beans, and since they are commonly confused, further brief comment on their differentiation is given. In the course of investigation of the remarkable resistance of navy bean starch to enzymatic digestion(11) enzyme inhibitors were detected in small amounts while working with the fatty fractions of both navy and soy beans(12). It was found that the inhibitors are much more abundant in aqueous extracts and that, although part of the trypsin inhibitor fraction of soy beans(13) is soluble in 60% alcohol, soy beans also contain an alcohol insoluble fraction(14). The globulin soy bean fraction later crystallized by Kunitz(15) corresponds with the latter(16) and the former is probably the fraction of Ham and Sandstedt(17) which is soluble in alcohol and lost on dialysis. The NTI(13,16) is soluble in 60% alcohol and is not crystallized by the method applicable to the GSTI. The NTI has the properties of a proteose: it precipitates in cold 0.25% nitric acid, redissolves with heat and reprecipitates on cooling, is soluble in 60% alcohol, precipitates in 0.5 saturated $(NH_4)_2SO_4$, and is more soluble in hot trichloroacetic acid than in cold. It dissolves in buffer solutions more readily than does the GSTI. The present NTI preparations give positive biuret, ninhydrin and Millon tests. The proteose soy bean trypsin inhibitor has similar properties; however, present preparations give a negative Millon's test and since it inhibits trypsin much less actively than do the GSTI or NTI it has been

used in this laboratory less frequently. The NTI and proteose soy bean trypsin inhibitor both inhibit chymotrypsin much more actively than does the GSTI.

Summary. Soy and navy bean trypsin inhibitors considerably reduce the incidence of fatal trypsin embolism in rabbits with the retention of marked thromboplastic activity by the inhibitor-enzyme complex. The thromboplastic effect is observed with little or no proteolytic activity and the relative protection against embolism is apparent even with extensive proteolytic activity. Similar observations were made with the bean trypsin inhibitors and hypochlorite-treated trypsin, the latter having been shown earlier to have considerably less hypotensive effect than untreated trypsin. The differentiation of the soy and navy bean trypsin inhibitors is briefly considered and an improved method of preparing the navy bean inhibitor is given.

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Distribution and Excretion of Radioactivity after Administration of Morphine-N-methyl C^{14} to Rats.* (21145)

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Our knowledge of the tissue distribution and excretion of morphine is limited because available analytical methods are not sensitive enough to determine tissue levels after the administration of a single therapeutic dose. The synthesis of morphine-N-methyl-C^{14}(1) has made it possible to follow the drug through the body using tracer technics. This report deals with tissue distribution and excretion of carbon-14 in rats after administration of minimal analgetic doses, as well as an investigation of N-demethylation of morphine *in vitro*.

Methods. In vivo studies: Adult Wistar-type rats of both sexes weighing approximately 200 g were given 5 mg/kg of suitably diluted morphine-N-methyl-C^{14}HCl subcutaneously. This constituted a minimal analgetic dose in the animals studied. The carbon-14 in the tissues, excreta and expired air was determined as previously described(2). For biliary tract excretion studies a rat was anesthetized with ether and a polyethylene catheter was inserted into the common bile duct through an uppermidline abdominal incision. The abdomen was closed, the animal was placed in a restraining cage and labeled morphine was administered after the animal had fully recovered from the anesthetic and bile was flowing freely.

Six female rats were given a total of 45 mg cyclopentyl testosterone propionate in three 15 mg subcutaneous doses over a 38-day period prior to studies on the effect of androgens. Two rats were made tolerant to morphine by daily injections for 7 weeks beginning

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