trol samples of blood and lymph were free of phage.

Samples of small intestine contents of 3 rats were taken after collection of blood and lymph was completed. Titration indicated that phage was not being inactivated by intestinal fluids, the counts ranging from 95 to 100% of the count of the phage suspension introduced.

These results clearly demonstrate that the primary path of translocation of coli phage T_1 through the intestinal walls is into the intestinal lymph rather than directly into the intestinal blood. These findings agree with those for bacteria and large protein molecules.

The number of particles appearing in the lymph is not impressive, considering the numbers administered. However, the fact that translocation occurs at all may be of significance in the pathogenesis of certain infections, allergic states and intoxications(5).

Summary. In the rat, coli phage strain T_1 has been shown to be translocated from the lumen of the intestine to the blood *via* the regional lymphatic system.

We wish to thank Mr. James Ng for excellent and painstaking assistance in the surgical aspects of this work.

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Inhibition of Coagulase Reaction of Pathogenic Staphylococci by Heparin *in vitro*.* (27147)

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Since the first description of staphylocoagulase by Loeb in 1908, numerous attempts have been made to ascribe a role to this reaction in the pathogenesis of staphylococcal infections and to link the process with that of normal human clotting, but no definitive answers have been given to either of these problems. It was felt that demonstration of significant inhibition of the coagulase clotting mechanism by a known thrombin inhibitor would provide both a key to the sight of action of coagulase and a useful tool with which to define its role in pathogenesis of staphylococcal infections.

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Materials and methods. Overnight broth cultures (Nutrient or Trypticase Soy Broth, Difco) of coagulase positive staphylococci were employed as a source of coagulase. Staphylococci of various bacteriophage types, isolated from 30 patients were utilized. Standardized Normal Human Plasma (Dade), Coagulase Rabbit Plasma (Baltimore Biological Laboratories), fresh human plasma, or fresh rabbit plasma was used to assay coagulase activity. Heparin (Upjohn) was employed as the thrombin inhibitor with all strains. In addition, heparin (phenol-free and Organon) was used with strains HE and 17262. The 0.5% phenol in commercial heparin did not interfere with growth of staphylococci in the assay system when the heparin was diluted 1:10 in 0.85% NaCl. Furthermore, Elek(1) has reported that coagulase

TABLE I. Coagulase Clotting Time (in Min.) of Strains of Staphylococci in Unheparinized and Heparinized Plasma from 4 Sources.

Staphylococcus strain	Source of plasma	Clotting time: Saline controls	Heparinized plasma: Heparin concentrations, mg/cc .14	.08	.07	.035	.018	.009
HE	SNP	60	>300*		>300	>300	300	270
17262	"	195	280*		>280	270	225	250
17473	"	45		>200				
17487	"	135		>200				
17499	"	45		>200				
17500	"	30		120				
17511	"	35		185				
17512	"	65		>200				
17523	"	35		180				
17524	"	45		190				
17535	"	35		>200				
17536	"	50		>200				
HE	Fresh rabbit	30	46†		46	69	38	30
HE	*Idem*	30	70*		70	70	70	70
11606	"	35	110					
17582	"	105	>150					
17448	"	30	130					
17590	"	25	55					
17486	"	30	105					
17578	"	55	130					
17613	"	30	75					
17473	"	40	>180					
17487	"	140	>180					
17500	"	60	>180					
17511	"	30	>180					
17512	"	40		>180				
17523	"	100		>180				
17524	"	30		>180				
17535	"	40		>180				
16923	Fresh human	40	125		90	55	50	50
16924	*Idem*	30	300		95	75	53	39
17094	"	57	300		300	110	80	70
17049	"	60	300		300	140	105	160
16923	Commercial rabbit	30	40		40	40	40	40
17476	*Idem*	30	180					
17489	"	125	220					
17501	"	55	220					
17513	"	110	220					
17525	"	55	180					
17537	"	25	125					

* Heparin, Organon. † Heparin, phenol-free.

loses no activity in presence of 0.1% phenol.

An overnight broth culture of staphylococcus was shaken to a uniform density. Twofold dilutions were made of 0.1 cc heparin in 0.85% saline (1.0 mg heparin/cc). As a control 0.1 cc of 0.85% saline was used. To these tubes 0.5 or 1.0 cc of 1:10 plasma in isotonic saline and 0.1 cc of the overnight broth culture were added. The resultant heparin concentrations varied from 0.14 to 0.009 mg/cc. Later in analysis of various strains of staphylococci, only one heparin concentration, 0.14 or 0.08 mg/cc was employed. Experiments were performed in duplicate and the tubes examined at 5-minute intervals for coagulation. Duplicates agreed within 10 minutes and results are averages of these values.

Since heparin is a weak acid, the pH of heparinized plasma and control mixtures was

determined. The pH of the mixture containing the greatest concentration of heparin was 6.4 while that of the saline control mixture was 6.6. This degree of difference was not considered significant. In other experiments, no inhibition of staphylococcal growth was observed with heparin alone.

Results. (1) *Standardized normal human plasma.* When varying concentrations of heparin preparations were mixed with strains of coagulase positive staphylococci and commercially available standardized human plasma, clotting times were prolonged from 55 to greater than 240 minutes (see Table). Heparin did not completely inhibit clotting, since in all tubes a clot was observed after 6-24 hours.

(2) *Freshly prepared rabbit plasma.* Similar results were obtained when fresh citrated rabbit plasma was used. In screening several strains against heparin 0.08 mg/cc, utilizing 1.0 cc of 1:10 citrated plasma in isotonic saline, no significant difference in coagulation times was noted. However, if only 0.5 cc of plasma were used in control and test sets, thus increasing heparin concentration to 0.14 mg/cc, a marked prolongation of coagulation times was observed. To determine the feasibility of *in vivo* studies, a rabbit was heparinized *in vivo* to approximate a blood concentration of 0.02 mg commercial heparin/cc. Then the rabbit was bled at the time of maximum anticoagulant effect. The plasma was separated with citrate, and tested simultaneously against the same rabbit's unheparinized plasma as a control. Utilizing staphylococcus strain HE, unheparinized plasma controls clotted in 35 minutes, while *in vivo* heparinized plasma clotted at 70 minutes.

(3) *Fresh human plasma and commercial rabbit plasma.* Similar results were again obtained when tested with 11 strains of staphylococci.

Irrespective of type of plasma, a qualitative and quantitative difference was noted in the physical character of clots formed in presence or absence of heparin. Without heparin, clots were large and bulky, comprising ⅔ to ¾ of the volume of mixtures. They did not fragment on gentle shaking. On the other hand, clots formed in presence of heparin were smaller and filamentous, occupying only ¼-½ of the volume of mixtures. They fragmented easily on shaking.

Frequently, prior to formation of a definitive clot a fine flocculation was observed. The appearance of this phenomenon correlated well with the established end point—*i.e.,* formation of a large clot or multiple small clots greater than 2.0 mm in size—in all cases except fresh rabbit plasma in which flocculation may appear first in the heparinized plasma.

Discussion and conclusions. Prolongation of coagulase-clotting time in presence of heparin assists in relating the coagulase reaction to the normal blood clotting mechanism. Elek (1) states that "coagulase-thrombin", a combination of coagulase and plasma "accessory factor," will clot purified fibrinogen suggesting an identity with naturally occurring thrombin. However, he discounts this possibility on the basis of reports of Walston(2), Rigdon(3,4) and Miale(5) stating that heparin, a known thrombin inhibitor, fails to inhibit the coagulase reaction *in vitro.* In reviewing the work of these authors and in preliminary experiments, it was felt that the effect of heparin upon coagulase reaction had not been adequately tested.

Walston and Rigdon had either failed to describe the source of their plasma or record the frequency of observation for clotting and character of clots, and had compared clotting times of coagulase in citrated or oxalated plasma with heparinized plasma except in a few instances. These studies would have been more satisfactory if the effect of coagulase on citrated plasma had been compared more extensively with its action on citrated plasma to which heparin had been added.

Miale's experiments more closely paralleled these, though a sterile, cell-free filtrate of broth cultures was employed as a source of staphylocoagulase, and Na-oxalate was used in preparation of plasma. The influence of these differences has not been evaluated. Miale states neither the source and composition of his heparin solution nor the frequency of observation of his plasma for clotting. More subtle differences in clotting times such as noted with strain 16923 and commercial rabbit plasma could be overlooked.

The results presented here suggest a similarity of "coagulase-thrombin" and thrombin. In addition, prolongation of coagulase clotting time by heparin may provide a useful tool in studying the role of coagulase in pathogenesis of staphylococcal infections. The possible influence of heparin upon staphylococcal infections was suggested by the studies of Borgstrom *et al.*(6) who found that it made experimental staphylococcal skin infection more amenable to treatment with penicillin.

Summary. Heparin has been shown to prolong coagulase clotting time of 30 strains of staphylococci. This finding suggests that coagulase may interact with an "accessory factor" of plasma to produce a substance analogous or identical to thrombin.

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Antifungal Activity of Decanoic Hydroxamic Acid. (27148)

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During an investigation of the pharmacologic properties of hydroxamates of certain carboxylic acids, it was noted that decanoic hydroxamate had a marked inhibitory effect on growth of certain fungi. This paper is a report of *in vitro* and *in vivo* studies of this compound.

Methods. Decanoic hydroxamic acid (DHA) was synthesized by the method previously described(1) and was recrystallized from 95% ethanol by addition of water. Assays of growth inhibition were carried out in liquid media, using Bacto-nutrient broth for bacteria, Kelley's broth(2) for *Cryptococcus neoformans, Blastomyces dermatitidis, Sporotrichum schenkii,* and *Histoplasma capsulatum,* and Bacto-Sabouraud broth for filamentous fungi and *Candida albicans.* Measurement of growth of the yeast-like organisms and bacteria was done turbidimetrically in a Coleman spectrophotometer; dry weight determinations were made of shaken cultures of the filamentous fungi. The former were grown at 37°C and the latter at room temperature. For respirometric studies a 24-hour broth culture of *C. albicans* grown at 37°C was washed with 0.1 M phosphate buffer, pH 7.4, and finally made to a 2.0% suspension (v/v) in this buffer. One ml of this was used in each Warburg vessel. The center well contained 0.2 ml 20% KOH to absorb CO_2. The final reaction volume was 2.0 ml and temperature was 37°C.

Results. Some degree of specificity of the inhibitory action of DHA against both filamentous and yeast-like fungi was evident (Table I); concentrations required for inhibition of certain bacteria were up to 20 times those required for fungi. Even though the hydrogen ion concentration of Sabouraud broth was about 1 pH unit lower than nutrient broth, this pattern bears some similarity to the results obtained by Wyss *et al.*(3) and Prince(4). The former authors, in their survey of antifungal properties of fatty acids, tested both fungi and bacteria at the same hydrogen ion concentrations. Whereas they found 2 dermatophytes to be inhibited by