smears of the blood continued to show *Bartonella canis.*

On February 5, 1944, Dog 2 was inoculated with 5 cc of whole blood taken from Dog 1. On that date the concentration of hemoglobin was 11.6 g per 100 cc of blood. Previous studies of stained smears of the blood of this animal had not revealed *Bartonella canis.* On February 9, 1944, the dog's spleen was removed. Stained blood smears and the concentration of hemoglobin were studied at weekly intervals. On March 3, 1944, the erythrocytes showed Bartonella bodies and by March 30, 1944, the hemoglobin had decreased to 7.4 g per 100 cc of blood. The hemoglobin continued to decrease and on April 20, 1944, the concentration was only 3.2 g per 100 cc of blood.

Dog 3, which had been splenectomized on September 5, 1939, was inoculated with 5 cc of whole blood from Dog 1 on March 3, 1944. The results were similar to those obtained in the case of Dog 2 and need not be recorded in detail.

The demonstration of organisms that had the morphologic characteristics of *Bartonella canis,* the transmission of the infection to other dogs with resultant anemia, and the demonstration of the organisms in their blood confirmed the diagnosis of spontaneous bartonellosis.

Summary. After splenectomy had been performed on a dog severe anemia developed and stained smears of the blood revealed organisms that had the morphologic characteristics of *Bartonella canis.* The disease was transmitted to two other dogs, confirming the diagnosis of bartonellosis.

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Effect of Intravenous Injection of Trypsin on the Blood Coagulation Time in Hemophilia.

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Suitable quantities of trypsin will reduce to normal, *in vitro,* the prolonged clotting time of hemophilic blood.[1] The intravenous injection of trypsin into a hemophilic patient has never been attempted, chiefly because experiments on animals have shown that trypsin is toxic and causes intravascular coagulation.[2,3]

However, the toxicity of trypsin is greatly reduced by injecting it slowly. Thus, crystalline trypsin*[4] dissolved in 200 to 300 ml of physiological saline was injected intravenously into 3 hemophilic patients and its effect on the coagulation time observed.

The coagulation time was measured at 37°C on duplicate samples of 2 ml of blood in chemically clean test tubes, 9 to 10 mm in diameter, previously rinsed with physiological saline. The end point is reached when the tube can be completely inverted without spilling the blood.[5]

The results (Table I) show that a significant shortening of the coagulation time followed the injection of 300 mg of trypsin in 19 minutes and of 325 mg in 20 minutes respectively in 2 different hemophilic patients. The coagulation time returned to the initial value after one hour. Observation 11 (Table I) shows that no effect on the coagulation time

1 Tyson, L. T., and West, R., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 494.

2 Rocha E Silva, M., *Arch. Path.,* 1942, **33**, 387.

3 Tagnon, H. J., *J. Clin. Invest.,* in press.

* Obtained from the Plaut Research Laboratories, Bloomfield, N.J. This preparation contains about 80% magnesium sulphate.

4 Northrop, J. H., *Crystalline Enzymes,* Columbia University Press, 1939, page 135.

5 Patek, A. J., and Stetson, R. P., *J. Clin. Invest.,* 1936, **15**, 531.

TABLE I.
Effect of Intravenously Injected Trypsin on Clotting Time of Hemophilic Blood.

Observation No.	Subject	Trypsin (mg)	Duration of injection (min)	Clotting time* (min—37°C)		
				1	2	3
1	SM.	2	33	88	93	
2	SM.	1	3	99	92	
3	SM.	30	28	125	109	
4	Roc.	40	36	136	124	
5	Roc.	60	28	153	166	
6	Roc.	120	45	109	117	
7	SM.	120	22	67	53	
8	SM.	250	20	90	54	88
9	SM	300	19	*92*	*30*	*91*
10	Roc.	325	20	*104*	*24*	*92*
11	T.K.	300	120	69	78	70

* 1. Before injection.
2. Immediately after the end of injection.
3. One hour after the end of injection.

TABLE II.
Effect of Trypsin on Clotting Time of Hemophilic Blood *in Vitro*.

Test tube No.	Trypsin (mg in 0.1 ml of saline)	Hemophilic blood* (ml)	Clotting time (min room temp.)
1	2	2	13
2	2	2	15
3	1	2	8
4	1	2	8
5	0.5	2	8
6	0.5	2	8
7	0.25	2	*5*
8	0.25	2	*5*
9	0.1	2	7
10	0.1	2	9
11	0.05	2	12
12	0.05	2	15
13	0	2	72
14	0	2	93

* From patient T.K.

followed the injection of 300 mg during a period of 120 minutes.

No toxic manifestations, local or general, were observed during or after the injections.

Table II shows that *in vitro* the optimal quantity of trypsin effective on the coagulation time of 2 ml of hemophilic blood was 0.25 mg. A significant effect was also observed with from 1.0 to 0.05 mg. Assuming the blood volume of the patients to be about 5000 ml, there seems to be a definite correlation between the effect of trypsin *in vivo* and *in vitro*. On this basis, a dose of 300 mg of trypsin should be expected to produce an effect *in vivo*.

Larger doses of trypsin were not tried because the toxic dose is probably very near the dose most effective on the clotting time.[3] For the same reason it was felt that normal subjects should not receive injections of trypsin in such doses as produced a distinct shortening of the coagulation time in hemophilic patients.

The action of intravenously injected trypsin, as described here, is of short duration (less than 1 hour) (Table 1). Its therapeutic

effectiveness in hemophilia thus does not compare with transfusion of either fresh blood or plasma. Furthermore, the toxicity of trypsin is great and extreme caution is advised in administering it intravenously. However, further investigations may be able to make its use safer and more effective.

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Biotin-Pantothenic Acid Interrelationship in Rats Fed Succinylsulfathiazole.

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The feeding of so-called "purified" rations containing sulfaguanidine or succinylsulfathiazole to rats has a marked depressing effect upon growth; an inhibition which can be counteracted by the feeding of liver extracts.[1,2] Daft and co-workers[3] reported that this deficiency syndrome was ascribable in part to a lack of biotin. The biotin deficiency was not progressive, due in all probability to the coexisting lack of folic acid. These findings were confirmed by Nielsen and Elvehjem[4] and by Martin.[5] The only other means of producing biotin deficiency in the rat is either by the feeding of a purified diet containing egg white or a purified ration with casein as the source of protein but supplemented with an avidin concentrate: the deficiency state thus produced progresses and leads to the ultimate death of the animal.

Wright and Welch[6,7] have reported that folic acid and biotin are necessary for the utilization of pantothenic acid by rats receiving purified diets containing succinylsulfathiazole. Animals fed this type of ration gave evidence of pantothenic acid deficiency despite the inclusion of adequate amounts of this vitamin in their diet. Even parenteral administration of pantothenic acid failed to restore the levels of this vitamin in the liver to the normal. West *et al.*[8] observed changes usually associated with a lack of pantothenic acid in rats maintained on a sulfapyridine containing low protein diet. However, the feeding of excess pantothenic acid cured these deficiency manifestations.

In the course of an investigation dealing with the influence of succinylsulfathiazole upon the utilization of pantothenic acid it was noted that signs of biotin depletion were much more marked in rats receiving the pantothenic acid deficient diet than in controls supplied with this vitamin. At a time when the rats receiving pantothenic acid showed only incipient manifestations of biotin deficiency, namely, spectacled eyes and sparse mole-like fur, the rats fed the pantothenic acid deficient diet showed a more advanced state of biotin depletion such as sore mouth, ventral alopecia and encrustations. These animals also had the porphyrin caked whiskers and pattern greying associated with a lack of pantothenic acid. Curative therapy with biotin was initiated at a time when the rats were failing rapidly from a lack of folic acid. Although some improvement was seen, weight losses were continuous and only incipient

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3 Daft, F. A., Ashburn, L. L., and Sebrell, W. H., *Science*, 1942, **96**, 321.

4 Nielsen, E., and Elvehjem, C. A., *J. Biol. Chem.*, 1942, **145**, 713.

5 Martin, G. J., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 353.

6 Wright, L. D., and Welch, A. D., *Science*, 1943, **97**, 426.

7 Wright, L. D., and Welch, A. D., *J. Nutrition*, 1944, **27**, 55.

8 West, H. D., Jefferson, W. C., and Rivers, R. E., *J. Nutrition*, 1943, **25**, 471.