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Haemobartonella tyzzeri in Colombia.

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This paper represents the results of our studies on splenectomized guinea pigs from Colombia, in relation to bartonella infection.

Weinman and Pinkerton[1] described a new species of bartonella from the guinea pig. It was called *Bartonella tyzzeri.* Later Tyzzer and Weinman,[2] on the basis of striking differences between the human and animal bartonellas, proposed a division of the genus Bartonella into Bartonella *sensu strictu* and Hæmobartonella, the latter to include all animal bartonellæ known at the time. The guinea pig parasite thus becomes *H. tyzzeri.* The organism was found in Peru in 4 of 23 splenectomized guinea pigs. Infected red cells appeared 5 to 9 days after operation and remained visible for a period varying between a few days and a month. *H. tyzzeri* appears as rods 1.4 to 4 μ long and 0.25 μ wide. The surface of the rods sometimes shows granular swellings. The poles are frequently enlarged. Short rods, averaging 0.8 by 0.2 to 0.3 μ, may also occur. Round, coccus-like forms may be observed. Sometimes the parasite takes a bipolar stain.

Of 20 locally obtained Colombian guinea pigs which we had occasion to splenectomize, one was naturally infected with a bartonella morphologically identical with *H. tyzzeri,* Weinman and Pinkerton. In this infected animal, the parasites appeared on the 8th day after operation. They continued to appear until the 28th day when the guinea pig died accidentally.

The parasite could be transmitted to 2 out of 3 splenectomized, bartonella-free guinea pigs, after intraperitoneal injection of 0.5 cc of infected blood. In one of these 2 guinea pigs, bartonellas appeared in the peripheral blood 5 days after the injection and were visible during two days; in the other guinea pig the parasites appeared on the 19th day following the injection; 35 days later they were still present in the blood stream.

1 Weinman, D., and Pinkerton, H., *Ann. Trop. Med. and Parasitol.,* 1938, **32**, 215.

2 Tyzzer, E. E., and Weinman, D., *Am. J. Hygiene,* 1939, **30**, Sec. B, 141.

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In vitro Effect of Tyrothricin and Tyrocidine Hydrochloride on Polymorphonuclear Leucocytes.

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This paper reports the effect of tyrothricin and tyrocidine hydrochloride upon exudative rabbit polymorphonuclear leucocytes and upon their phagocytic action *in vitro.* In high concentrations these substances caused cytoplasmic and nuclear disintegration; in lower concentrations altered staining reactions were observed. When there was no apparent microscopic injury to the cells, phagocytosis of pneumococci occurred as expected. It was also found that the presence of serum afforded some protection for the cells from the effects of these substances.

Dilutions of tyrothricin and tyrocidine hydrochloride were mixed with constant amounts of suspensions of a Type I mucoid strain of pneumococcus and of rabbit leucocytes, which were obtained according to the

TABLE I.

Effect of Tyrothricin and Tyrocidine Hydrochloride on Rabbit Leucocytes.

Rabbit leucocytes	Tyrothricin: Undil.	1/40	1/400	1/4000	1/40,000	1/400,000	1/4,000,000	Saline
In absence of serum	+++	++	++	+	0	0	0	0
*With normal rabbit serum	++	+	0	0	0	0	0	0
*With antipneumococcic serum	+++	++	+	+	+	0	0	0
	Tyrocidine hydrochloride: 1.0 mg	0.1 mg	0.01 mg	0.001 mg	0.0001 mg			Saline
*With antipneumococcic serum	+++	++	+	0	0			0

0—No disintegration.
+—Partial cytoplasmic disintegration.
++—Partial cytoplasmic and nuclear disintegration.
+++—Entire cytoplasmic and nuclear disintegration.
*Serum diluted 1:40; all concentrations are expressed as final concentrations.

TABLE II.

Effect of Tyrothricin and Tyrocidine Hydrochloride on Phagocytosis and Pneumococci.

Pneumococci Approximately 2,400,000,000 per 0.1 ml	Serum 1/40	Tyrothricin: Undil.	1/40	1/400	1/4000	1/40,000	1/400,000	1/4,000,000	Saline
	Normal rabbit serum								
	% of phagocytosis	0	0	6	6	6	6	6	6
	Amt of lysis of pneumococci	++	++	+	0	0	0	0	0
	Antipneumococcic serum								
	% of phagocytosis	0	80	80	100	100	100	100	100
	Amt of lysis of pneumococci	++	+	+	0	0	0	0	0
		Tyrocidine hydrochloride: 1.0 mg	0.1 mg	0.01 mg	0.001 mg	0.0001 mg			Saline
	Antipneumococcic serum								
	% of phagocytosis	0	100	100	100	100			100
	Amt of lysis of pneumococci	0	0	0	0	0			0

0—No lysis.
+—Partial lysis.
++—Complete lysis.
All concentrations are expressed as final concentrations.

method of Mudd and his coworkers.[1] The tyrothricin used was obtained from a culture, "B.G.", received from Dubos and prepared according to directions accompanying the culture.[2] The purified tyrocidine hydrochloride was supplied by Dr. W. F. Verway of Sharp and Dohme, Inc. These mixtures were shaken for 10 min at 37°C in the Boerner-Mudd apparatus[3] and smears were made, which were stained by a special

1 Mudd, S., Lucke, B., McCutcheon, M., and Strumia, M., *J. Exp. Med.*, 1929, **49**, 779.

2 Dubos, R. J., personal communication, Dec. 26, 1939.

3 Boerner, F., and Mudd, S., *Am. J. Med. Sci.*, 1935, **189**, 22.

phagocytic stain.[4] Suitable control experiments were conducted which showed that the media in which the tyrothricin was dissolved had no effect in the dilutions considered.

Table I shows the actions of tyrothricin and tyrocidine hydrochloride on leucocytes in the presence and absence of serum. This finding is typical of the results obtained in 20 instances.

Table II shows the effect of tyrothricin and tyrocidine hydrochloride upon the amount of phagocytosis taking place, recorded as the percentage of leucocytes containing pneumococci when 50 cells were observed, and also upon the pneumococci themselves, expressed by the amount of lysis occurring.

When normal leucocytes in saline suspension are treated with the phagocytic stain employed, the nuclei are stained red and the cytoplasm a purplish blue. It was noted that in some of the lower concentrations of tyrothricin and tyrocidine hydrochloride this staining reaction was reversed, the nuclei appearing blue and the cytoplasm red. The cells exhibiting these peculiar staining reactions were also noted to have ingested more pneumococci than those staining in the normal fashion.

The author wishes to express appreciation for suggestions made by Dr. David B. Lackman during the course of this work.

[4] Lucke, B., Strumia, M., Mudd, S., McCutcheon, M., and Mudd, E. B. H., *J. Immunol.*, 1933, **24**, 455.

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The Acute Toxicity of Choline Hydrochloride in Mice and Rats.*

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Although a number of isolated observations on the acute toxicity of choline have been recorded, no systematic studies are available. The relatively low toxicity of choline is illustrated by the statement of Mott and Halliburton[3] that "We have never succeeded in killing an animal by injection of choline or choline hydrochloride." However, the lethal dose of choline has been estimated for several species as shown in the accompanying text table and the generality has been established that animals either die promptly (within 20 min) from the effects of choline or recover, apparently without permanent injury.

No observations on rats are available.

In the tests reported here, choline hydrochloride (Eastman Kodak Co.) was given in aqueous solutions as indicated (Table I). Death, when it occurred, followed promptly —for large doses within 2-4 min in some of the animals. The symptoms were those previously described by many investigators; in

Toxicity of Choline Hydrochloride.

Species	Lethal dose	Route	Reference
Mice	700 mg/kg	Subcutaneous	1
Cats	35 mg/kg	Intravenous	1,2
Rabbits	1 g/kg	Rectally	5
	0.11 cg/kg	Intravenously	5
	1 g/kg	Subcutaneously	5
Frogs, large	0.1 g	?	4
Frogs, small	0.05 g	?	4

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[1] Arai, K., *Arch. ges. Physiol.*, 1922, **193**, 359.

[2] Lohmann, A., *Arch. ges. Physiol.*, 1907, **118**, 215.

[3] Mott, F. W., and Halliburton, W. D., *Trans. Roy. Soc. Lond.*, B, 1899, **191**, 211.

[4] Boehm, R., *Arch. Exp. Path. Pharm.*, 1885, **19**, 87.

[5] Dreyfus, L., *C. N. S. B.*, 1920, **83**, 481.