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Relation of Acetyl Choline to Anaphylactic Shock in the Rabbit.*

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The release of a histamine-like substance into the blood stream during anaphylactic shock, as first suggested by Dale and Laidlaw,¹ has been fairly well established. Dragstedt has isolated a histamine-like substance from the blood stream and thoracic duct of intact animals during anaphylaxis;² and this substance has been demonstrated in the perfusate of an isolated guinea pig lung during anaphylactic shock.^{3, 4}

Discrepancies between the syndrome of anaphylaxis and the pharmacological action of histamine have led to the search for other "humors" to explain the former phenomenon. Schittenhelm and his coworkers showed that the serum potassium rose markedly in rabbits and dogs during anaphylactic shock.⁵ Went and Lissák claim to have isolated choline from the perfusate of an isolated guinea pig heart during anaphylactic shock.⁶

Wenner and Buhrmester report that acetyl choline can be detected in rabbit's heart blood during anaphylaxis.⁷ This liberation of acetyl choline in the blood was measured both by Fühner's sensitized leech preparation,⁸ and by the effect on a cat's blood pressure of an extract made according to Bischoff and Kapfhammer's method.⁹ Although the leech technic is more sensitive, Wenner and Buhrmester noted that in some experiments, with no measurable concen-

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¹ Dale, H. H., and Laidlaw, P. P., *J. Physiol.*, 1910, **41**, 318.

² Dragstedt, C. A., and Gebauer-Fuelnegg, E., *Am. J. Physiol.*, 1932, **102**, 512, 520.

³ Bartosch, R., Feldberg, W., and Nagel, E., *Pflüger's Arch.*, 1932, **230**, 129, 674; 1933, **231**, 616.

⁴ Spinelli, A., *Boll. Sez. ital. Soc. int. Microbiol.*, 1932, **4**, 257.

⁵ Schittenhelm, A., Erhardt, W., and Warnat, K., *Klin. Wochenschr.*, 1927, **6**, 2,000.

⁶ Went, S., and Lissák, K., *Arch. Exp. Path. u. Pharm.*, 1936, **182**, 509.

⁷ Wenner, W., and Buhrmester, C., *J. Allergy*, 1937, **9**, 85.

⁸ Fühner, H., *Arch. Exp. Path. u. Pharm.*, 1917, **82**, 81; *Biochem. Z.*, 1918, **92**, 347.

⁹ Bischoff, C., and Kapfhammer, J., *Z. Physiol. Chemie*, 1930, **191**, 179.

tration of acetyl choline could be detected by this means, Bischoff and Kapfhammer extracts would show "considerable amounts of acetyl choline." Concentrations of 1:1,000,000 to 1:10,000,000 were reported for the final concentrated extract of heart's blood of rabbits dying in shock.¹⁰

Although normal rabbit's blood will not cause contraction of the sensitized leech, by the Bischoff and Kapfhammer method it will show an acetyl choline concentration of about 0.05 gamma per cc, or 1:20,000,000.¹¹ Since Wenner and Buhrmester's figures are for a concentrated extract, and not for whole blood, it would seem that the concentration of acetyl choline found by them was within normal limits for this method. It is to be noted that these workers made no attempt to inhibit the very rapid *in vivo* destruction of acetyl choline by the blood esterase.

Experimental. Detection of Acetyl Choline in the Blood of the Shocked Animal: A group of rabbits was sensitized to whole egg albumen according to Grove's method.¹² The animals were injected with 1 cc of 50% egg albumen intravenously, followed 5 and 10 days later by 5 cc of egg albumen intraperitoneally; and then by 7 daily intraperitoneal injections of 1 cc each. Seven days after the last injection the rabbits were given 1.5 cc of antigen subcutaneously and were finally shocked on the 31st day with 3 to 5 cc of 50% egg albumen given intravenously.

Of 16 rabbits, 11 survived the course of sensitization, and were shocked a total of 12 times. Three animals were shocked without previous physostigmine injection; these animals showed 2 severe, and 1 mild shock. In 9 rabbits the injection of egg albumen was preceded by 0.15 mg/kilo of physostigmine intravenously. The physostigmine was administered to prevent the otherwise rapid destruction of acetyl choline by the blood choline esterase. Each of these 9 was shocked severely enough to die following the added insult of the withdrawal of heart's blood. At the first symptoms of shock, heart's blood was withdrawn into a syringe containing a few drops of 8% solamine fast pink, an analogue of the anticoagulant dye chlorazol fast pink, to which had been added physostigmine solution, 1:100,000. The blood was then added to a leech muscle preparation sensitized at room temperature with 1:100,000 physostigmine in frog Ringer's solution. Viability of the leech was always tested with known concentrations of acetyl choline; the effectiveness

¹⁰ Personal communication.

¹¹ Chang, H. C., and Gaddum, J. H., *J. Physiol.*, 1933, **79**, 255.

¹² Grove, Ella, *J. Immunology*, 1932, **23**, 101.

of the physostigmine in inhibiting the choline-esterase was tested by adding known amounts of acetyl choline to the eserinated heart's blood. In 11 of 12 experiments the leech was sensitive to at least 1×10^{-7} of acetyl choline; usually about 2 or 3×10^{-8} caused a vigorous contraction.

In no case did the leech muscle contract following the addition of the shocked rabbit's blood. On the contrary, relaxation of the leech occurred in some instances.

Summary. No direct evidence could be found to support the view that acetyl choline is released into the blood stream in detectable amounts in anaphylactic shock in the rabbit.

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Transmission of Endocarditis Lenta to Rabbits.

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Rosenbach¹ produced experimental bacterial endocarditis in dogs by passing an instrument down the right carotid artery to wound the valves. Most of his experiments on dogs and rabbits produced aseptic lesions but 2 of his dogs developed large vegetations on the aortic valve and numerous septic infarcts and petechial hemorrhages in various organs. Colonies of micrococci, apparently staphylococci, were demonstrated in these lesions. Ever since that pioneer observation there has prevailed the idea that some accessory injury of the endocardium is necessary in addition to the introduction of bacteria into the blood-stream, if one is to succeed in causing experimental endocarditis. Welch, Murdock and Ferguson² punctured the heart and then rotated the needle to produce a preliminary endocardial wound before introducing their bacteria intravenously or into the pulp of a tooth. In addition they resorted to accessory respiratory infection and dietary restriction to lower the resistance of their animals. In this way they succeeded in producing gross endocardial vegetations in 20% of their rabbits inoculated with endocarditis-

¹ Rosenbach, Ottomar, *Arch. f. exp. Path. und Pharm.*, 1878, 9, 1.

² Welch, Henry, Murdock, Thomas P., and Ferguson, John A., *J. Lab. and Clin. Med.*, 1936, 21, 1264.