13161

Effect of Trypsin upon Blood Histamine of Rabbits.

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It has been shown in previous experiments[1] that trypsin causes a contraction of various isolated smooth muscle structures such as the intestine of the guinea pig, rabbit, etc., and that this smooth muscle effect is characterized by a short latent period and by a subsequent desensitization, phenomena which are analogous to those seen when antigen is added to the bath containing the tissues of sensitized animals. To further amplify the analogy between the action of trypsin upon normal tissues and the action of antigen upon sensitized tissues, we have found that the addition of arginin or histidin to the bath will prevent or counteract the effects of trypsin just as it does the effects of antigen.[2] It has also been shown that the intravenous injection of trypsin into cats, dogs and rabbits produces a profound vascular shock similar in many respects to that of anaphylactic shock.[3] In addition, it has been shown[4] that the intravenous injection of trypsin into dogs leads to the liberation of histamine into the blood such as occurs in anaphylactic shock.[5]

Rose and Weil[6] found that the injection of antigen into sensitized rabbits leads to a reduction in the total blood histamine and this finding has been confirmed in our laboratory.[7] This apparently paradoxical behavior of the rabbit appeared to offer a suitable experiment by which the effects of trypsin could be compared further with those of antigen.

Crystalline trypsin,† in amounts varying from 3 to 7 mg per kilo, was injected via the marginal ear vein into 5 rabbits. Blood

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1 Rocha e Silva, M., *Arch. f. exp. Path. u. Pharm.*, 1940, **194**, 335.

2 Ackermann, D., *Naturwiss*, 1939, 515.

3 Rocha e Silva, M., *Arch. f. exp. Path. u. Pharm.*, 1940, **194**, 351.

4 Ramirez de Arellano, M., Lawton, A. H., and Dragstedt, C. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 360.

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

6 Rose, B., and Weil, P., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 494.

7 Dragstedt, C. A., Ramirez de Arellano, M., and Lawton, A. H., *Science*, 1940, **91**, 617.

† Plaut Research Laboratory preparation.

TABLE I.
Histamine Content and Leukocyte Count of Rabbit's Blood Before and After the Intravenous Injection of Trypsin.

Exp.	Before		After	
	W.B.C.	Histamine	W.B.C.	Histamine
1	—	5.2	—	0.4
2	9,200	3.5	5,200	0.4
3	12,700	6.0	5,200	2.0
4	6,500	9.0	3,200	4.5
5	12,200	1.5	4,100	0.1

Histamine values in micrograms of histamine acid phosphate per cc of blood.

was obtained by cardiac puncture before and after the injection and assayed for its histamine content by Code's method.[8] Leukocyte counts were also made. The results are shown in the accompanying table.

As noted in the table, there was a striking reduction in the total blood histamine in every case. There was also an associated marked reduction in the leukocyte count. Code[9] has presented evidence that from 70 to 100% of the total blood histamine of rabbits is contained in the white blood cells and also[10] evidence that the granular leukocytes are the major source. From the results indicated in the table, it is apparent that the reduction in total blood histamine cannot be accounted for entirely by the reduction in the total leukocyte counts. As an alternative explanation for the much greater reduction in histamine than the corresponding reduction in leukocytes was obtained by studying the effects of trypsin on rabbit's blood *in vitro,* no attempts to correlate the histamine changes with counts of the granular cells were made.

Katz[11] demonstrated that when antigen is added to blood from a sensitized rabbit *in vitro,* there is a release of a considerable fraction of the cellular histamine into the plasma. We have found trypsin to have a similar effect. Rabbit's blood was obtained by cardiac puncture, heparinized to prevent clotting, and divided into 3 portions. The total blood histamine was determined on the first sample, the second sample was centrifuged and the plasma histamine determined, trypsin was added to the third sample and it was then centrifuged and the plasma assayed for histamine. The addition of trypsin caused no change in the total blood histamine, while the plasma

8 Code, C. F., *J. Physiol.,* 1937, **89**, 257.
9 Code, C. F., *J. Physiol.,* 1937, **90**, 349.
10 Code, C. F., *J. Physiol.,* 1937, **90**, 485.
11 Katz, G., *Science,* 1940, **91**, 221.

histamine was doubled or trebled, indicating that there must have been a release of histamine from cells to plasma. There is no reason to doubt that a similar release of histamine from cells to plasma occurs when trypsin is injected into the intact animal. In the latter instance the plasma histamine undoubtedly diffuses rapidly into the various tissues so that the reduction in total blood histamine which results when trypsin is injected into rabbits is partially due to the removal of leukocytes from the circulating blood and partially to the diffusion of histamine from blood cells to plasma to tissues.

Summary. The intravenous injection of trypsin into rabbits results in a prompt reduction in the total blood histamine with an associated leukopenia. The addition of trypsin to heparinized rabbit's blood *in vitro* results in a shift of histamine from cells to plasma. In both respects the effects of trypsin are similar to those produced by antigen in sensitized animals.

13162

Relationship Between Osmotic Activity and Sodium Content of Gastric Juice.*

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Several investigators[1] have advanced evidence for the view that gastric juice consists of a mixture of component secretions. Hollander has postulated at least two such components, one a solution of hydrochloric acid isosmotic with blood and the other an alkaline fluid with an electrolyte distribution more or less similar to that of plasma.[2] It was his conclusion that the mixture of these two component secretions would have a total ionic concentration less than either of the "pure" components. Under such circumstances buffering would occur as represented by the following reactions:

$$(1)\quad H^+ + Cl^- + Na^+ + HCO_3^- \rightarrow Na^+ + Cl^- + \underset{\uparrow}{H_2CO_3}{}^{CO_2 + H_2O}$$

$$(2)\quad H^+ + Cl^- + Na^+ + Buffer^- \rightarrow H\ Buffer + Na^+ + Cl^-$$

*We are indebted to Mr. Charles Carr for carrying out the sodium determinations for this study.

1 Gamble, James L., and McIver, Monroe A., *J. Exp. Med.*, 1928, **48**, 837; Hollander, Franklin, *J. Biol. Chem.*, 1932, **97**, 585; Dienst, C., *Ges. Exp. Med.*, **33**, 718; Liu, A. C., Yuan, I. C., and Lim, R. K. S., *Chinese J. Physiol.*, 1934, **8**, 1.

2 Hollander, Franklin, *Am. J. Dig. Dis. and Nut.*, 1936, **3**, 651.