Summary. Typhus vaccine, consisting of killed rickettsiae from infected rat lungs prepared according to the method of Castaneda, protected guinea pigs against infective doses of virus injected intraperitoneally. Immunization was accomplished by the subcutaneous injection of the vaccine in as small a dose as 0.2 cc. The virus used in the preparation of the vaccine and for the test for immunity was the "L" strain of Mexican typhus.

11576

Inhibition of Tryptases by Heparin.*

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It has been demonstrated by Fischer[1] that the isoelectric point of proteins shifts towards the acid side in the presence of heparin. He concluded that the acidic groups of heparin combined with the basic groups of proteins, forming salt-like compounds. Since trypsin attacks negatively charged proteins very readily,[2] it might be expected that the addition of heparin would facilitate proteolysis by making the protein behave as a stronger anion (at constant pH). However, trypsin is also a protein, and it may be that heparin can alter its activity by combining with it. The results of this paper indicate that heparin *decreases* the proteolytic activity of serum tryptase and crystalline trypsin.

Two methods were used to study the inhibitory action of heparin: (1) the increase in the non-protein nitrogen which resulted from the digestion of casein by trypsin was followed by means of micro-Kjeldahl analyses; and (2) the rate of inactivation of thrombin by trypsin or serum tryptase ("progressive" antithrombin) was estimated by assaying the coagulant activity of the residual thrombin.[3]

A. *Action of Heparin on Casein + Trypsin.* Various amounts of a heparin preparation† were added to 5 cc portions of a 3% casein solution, buffered to pH 8.2 with M/10 phosphate. The final volumes were adjusted to 7 cc, the mixtures warmed to 38°, and 1 cc

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1 Fischer, A., *Biochem. Zeits.*, 1935, **278**, 133.

2 Northrop, J. H., *Crystalline Enzymes* (New York), 1939.

3 Glazko, A. J., and Ferguson, J. H., *J. Gen. Physiol.*, 1940, **24**, 169.

† Connaught Laboratories. The preparation assayed 110 units per mg.

(= 0.5 mg) of a solution of crystalline trypsin‡ was added to each sample. One cc portions were withdrawn by pipette after specified time intervals and added to 4 cc portions of 10% trichloracetic acid. The mixtures were shaken and allowed to stand a half-hour before filtration. One cc of each filtrate was taken for analysis by the usual micro-Kjeldahl technic. The results of this procedure, corrected for the variation in NPN due to the heparin-N, are given in Table I. It is evident that the rate of proteolysis is decreased in the presence of heparin. In a similar experiment where crystalline papain was used in place of trypsin, no significant inhibition was observed.

TABLE I.
Inhibition by Heparin of Tryptic Digestion of Casein.

Heparin (mg/cc)	Time of incubation in min. 10′	20′	40′	80′	160′
	mg NPN per cc				
5.00	.00	.00	.01	.01	.02
2.50	.08	.16	.27	.47	.71
1.25	.23	.49	.77	.93	1.14
0	.34	.65	.89	1.19	1.28

B. *Effect of pH on the Inhibition of the Tryptic Digestion of Casein.* In order to study the effect of hydrogen ion concentration on the antitryptic behavior of heparin, 1 mg/cc of heparin was added to a 3% casein solution (buffered with N/10 phosphate) and the pH was adjusted to give the values shown in Table II, the final volumes being made the same in each case. The pH measurements were made with a glass electrode and a Beckman pH meter. One cc (= 0.5 mg) of a solution of trypsin was then added to 5 cc portions of the heparin-casein mixtures at 38°, and the NPN was determined after various time intervals as described in the preceding section. The results are shown in Table II. The optimum pH for digestion under these particular conditions appears to be shifted to the acid side of

TABLE II.
Effect of pH on Heparin-Inhibition of Tryptic Digestion of Casein.

pH	Time of incubation in min. 0′	10′	47′
	mg NPN per cc		
11.40	.33	.32	.32
9.40	.31	.60	1.10
7.90	.32	.70	1.15
7.00	.31	.69	1.20
5.90	.32	.56	.94

‡ Plaut Research Laboratories, Bloomfield, N. J. The preparation contained about 14% protein, the rest being $MgSO_4$.

the optimum for the tryptic digestion of casein in the absence of heparin (approximately pH 8.4).

C. *Rate of Thrombin Inactivation by Trypsin and Serum in the Presence of Heparin.* In a recent publication[3] we have shown that the rate of inactivation of thrombin (thrombinolysis) by trypsin is directly dependent on the concentration of trypsin, and may be characterized by a first order rate constant. This value may be determined quite readily by using the coagulation time of fibrinogen as a means of thrombin assay. The following equation may be used, provided the coagulation time is inversely proportional to the concentration of thrombin over the range studied:[3]

$$k = \frac{2.3}{(t_2 - t_1)} \log_{10} \frac{y_2}{y_1},$$

where k is the reaction rate constant and y_1 and y_2 are the observed coagulation times obtained with samples taken at times t_1 and t_2. The values for k in Table III represent the fraction of thrombin inactivated in one minute at 38°.

Table III A shows the effect of heparin on the rate constant which is obtained when a given amount of trypsin is added to thrombin. Trypsin alone will not coagulate purified fibrinogen,[4] and therefore does not interfere with the clotting test. The thrombin used was prepared by activating the isoelectric precipitate obtained on acidifying diluted plasma to pH 5.3, and then isolating the thrombin by acetone precipitation.[5] The negative k value in one case is probably due to the activation of a trace of prothrombin by the trypsin.[4]

TABLE III.
Effect of Heparin on Thrombinolysis.

	Heparin (μg/cc)	$k \times 10^4$ per min
A. 0.30 mg trypsin added to 5 cc of thrombin. pH = 6.9	1300	—15
	650	7
	320	210
	160	250
	0	437
B. An "unstable" thrombin preparation pH = 7.6	1670	76
	835	89
	417	107
	0	220
C. 0.8 cc of serum added to 5 cc of thrombin. pH = 7.4	1.20	224
	0.60	320
	0.30	326
	0.15	315
	0	407

[4] Eagle, H., and Harris, T. N., *J. Gen. Physiol.*, 1937, **20**, 543.
[5] Astrup, T., and Darling, S., *J. Biol. Chem.*, 1940, **133**, 761.

Table III B shows the results obtained with an "unstable" thrombin to which heparin was added, and Table III C shows the effect of heparin in retarding the "progressive" destruction of thrombin by fresh serum.[3] Ample evidence has already been presented for the presence of a tryptase in serum.[3, 6, 7, 8]

These results are wholly consistent with the data in section A. Heparin apparently decreases the rate of thrombinolysis because it interferes with the thrombin-tryptase interaction. This inhibiting action must not be confused with the *"immediate" antithrombic action* of heparin (in the presence of the accessory plasma factor) in preventing the thrombin-fibrinogen coagulation reaction.[9] However, it may be quite significant in the *antiprothrombic* action of heparin, since we know that trypsin and tissue extracts (which presumably contain tryptases) are capable of overcoming the inhibitory action of heparin in the first phase reactions.[10]

Summary. Heparin inhibits the action of trypsin and serum tryptase. The significance of this phenomenon in the blood clotting mechanism is briefly discussed.

11577

Detoxication of Benzoic Acid by Glucuronic Acid in Humans. Rate of Detoxication.

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I. *Amount of Detoxication by Glucuronic Acid.* While there is definite evidence that in the pig, the dog and the sheep, the feeding of benzoic acid results in the elimination of hippuric acid and of benzoyl glucuronide,[1] showing detoxication with glycine and with glucuronic acid, in humans the results are conflicting. Brakefield,[2] for example, finds detoxication with glycine, but no detoxication

6 Collingwood, B. J., and McMahon, M. T., *J. Physiol.*, 1913-14, **47**, 44.

7 Schmitz, A., *Zeits. physiol. Chem.*, 1936, **244**, 89; 1937, **250**, 37.

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9 Glazko, A. J., and Greenberg, D. M., *Am. J. Physiol.*, 1940, **128**, 399.

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1 Csonka, F. A., *J. Biol. Chem.*, 1924, **60**, 545; Brakefield, J. L., and Schmidt, C. L. A., *ibid.*, 1926, **67**, 523.

2 Brakefield, J. L., *J. Biol. Chem.*, 1927, **74**, 783.