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.

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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.

10 Ferguson, J. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 33.

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.

whatever with glucuronic acid. Quick,[3] on the other hand, claims a detoxication with glucuronic acid, which may, under certain conditions, amount to as high as 10 to 12%.

In the experiments of Brakefield and Quick only 2 subjects were used. Furthermore, the method of estimating the glucuronide, by applying reduction procedures, is open to question.[4]

Our work is an attempt to clear up this problem. In this investigation, as in a previous one,[5] we used the method of Maughan, Evelyn and Browne[6] to determine urinary glucuronides and obtained results in normal control urines consistent with those of other workers. In

TABLE I.
Amounts of Glucuronic Acid Eliminated.

Subject	Amt ingested, g	Mg glucuronic acid per 24-hr sample Days 1	2	3	4*	5	6	7¶	Avg mg glucuronic acid per 24 hr in normal urine†	Increase in mg glucuronic acid on ingestion of indicated dose‡
A	5	482	484	469	733	604	690	613	478	255
B	5	378	558	456	1022	468	426	436	464	558
C	5	461	582	527	826	622	537	493	523	303
D	5	432	572	483	972	729	646	568	496	476
E	5		444	559	688	590	532	547	501	187
F	5	522	494	576	1241	595	500	449	531	710
G	5	357	417	485	736	304	647	455	420	316
H	5	535	300	509	636	312	407	574	448	188
I	5	546	859	488	1272	773	795	762	631	641
J	5	410	372	478	861	525	522		420	441
K	5	569	559	520	899	501	562	554	549	350
L	6§	366	352	405	896	445	422	358	374	522
M	6	762	797	765	1141	773	795	762	775	366
M	7	Avg Norm. = 775			1205				775	430
M	8	Avg Norm. = 775			1750				775	975

*Ingestion of benzoic acid at the beginning of the fourth day.

†These figures were obtained by taking the average of the first 3 days.

‡These figures were obtained by subtracting the average of the first 3 days from the figure of the fourth day which represents the day of ingestion.

§It was not found easy to ingest more than 5 g of benzoic acid without developing nausea, headache, and a tendency to vomit. This explains the fewer subjects. Three subjects ingested 8 g of benzoic acid; 2 vomited within 25 minutes. The third was able to retain the benzoic acid; the results of the analyses on his urine samples are given in the table.

¶The amounts of glucuronic acid recorded in the table for the fifth, sixth, and seventh days are given as a comparison with the first 3 days to show that one day following the ingestion of benzoic acid the urinary glucuronides tend to return to within the subject's normal range.

[3] Quick, A. J., *J. Biol. Chem.*, 1931, **92**, 65.

[4] Harrow, B., and Sherwin, C. P., *A Text-Book of Biochemistry*, 1935, p. 380.

[5] Wagreich, H., Kamin, H., and Harrow, B., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 468.

[6] Maughan, G. B., Evelyn, K. A., and Browne, J. S. L., *J. Biol. Chem.*, 1938, **126**, 567.

addition, we have also studied the rate of excretion of benzoyl glucuronide.

Experimental Procedure. Twenty-four-hour urine samples of normal male subjects on a normal diet were collected for 3 days. On the fourth day, the indicated amount of benzoic acid was ingested The benzoic acid was first neutralized with 0.35 M NaOH and diluted to 200 ml. The urine was collected during the following 4 days. The amount of glucuronide was determined in each 24-hour sample according to the method of Maughan, Evelyn, and Browne,[6] which makes use of the color developed with naphthoresorcinol and concentrated hydrochloric acid. The instrument used for analysis was a single-cell direct reading photoelectric colorimeter.

The results with 15 subjects are recorded in Table I.

Table II summarizes these results. It can be seen that an appreciable quantity of benzoic acid is excreted as the glucuronide in the human. The ingestion of from 5-7 g of benzoic acid gives rise to an excretion of about 5% in the form of its glucuronide. Greater percent detoxication is apparently obtained with 8 g of benzoic acid. However, we do not want to stress this point inasmuch as we cite one example. We have already pointed out our difficulty and for that matter, the difficulty of other investigators, in getting subjects to ingest more than 5 g of benzoic acid.

II. *Rate of excretion of the glucuronide.* Urines were collected in 3-hour intervals before and after ingestion of the benzoic acid. Two individuals were given 5 g; two, 6 g; one, 7 g; and one, 8 g.

Fig. 1 shows 3 typical curves. It can be seen that the maximum amount of detoxication by glucuronic acid occurs in the first 3 hours after ingestion; and that with 5 and 6 g of benzoic acid, practically complete detoxication occurs within 9 hours. With 8 g of benzoic acid, complete detoxication does not occur before about 15 hours. It is interesting to note that Quick,[3] using one subject who ingested 8 g of benzoic acid, and making determinations at one-hour intervals, obtained maximum excretion of glucuronides in 2 hours.

Summary. (1) Contrary to Brakefield, and in agreement with

TABLE II.
Increase in Glucuronic Acid as a Result of Feeding Benzoic Acid.

Amt of benzoic acid ingested g	No. of cases	Avg increase in glucuronic acid in mg	% detoxication
5	11	402	5.0
6	2	444	4.7
7	1	430	3.9
8	1	975	7.7

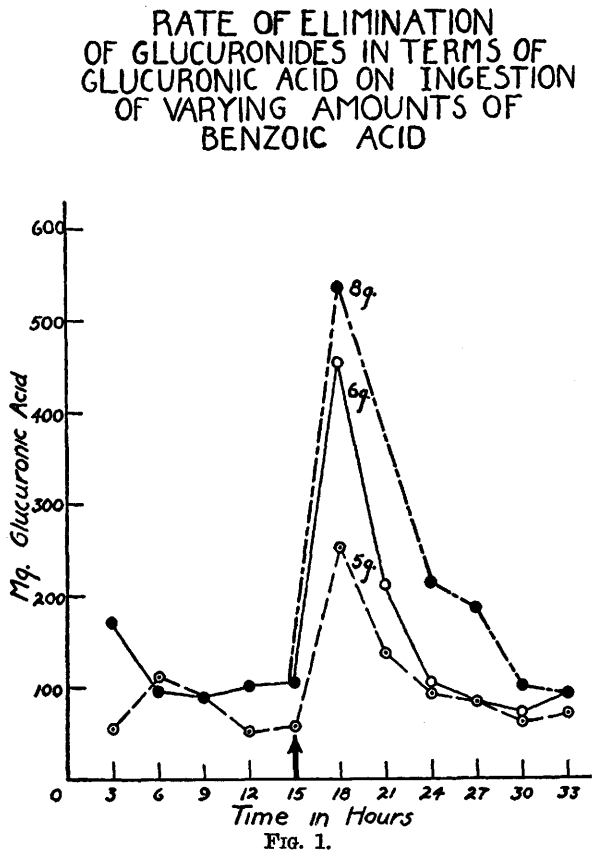

FIG. 1.

In each case, 5 three-hour normal samples of urine were taken. On the fifteenth hour, marked by an arrow on the graph, the benzoic acid was ingested.

The normals for the curves representing the 6 and 8 grams are the same because the same subject was used.

Quick, we find that in humans an appreciable quantity of benzoic acid is excreted as the glucuronide. The ingestion of 5, 6, and 7 g of benzoic acid leads to an excretion of some 5% in the form of its glucuronide. The ingestion of 8 g gives a higher figure (7.7%). (2) The maximum excretion occurs in the first 3 hours. Detoxication by glucuronic acid is complete in 9 to 15 hours, depending on the amount of benzoic acid ingested.