chloride or sodium in both distal and proximal segments. Administration of large doses of stable iodide, perchlorate or thiosulfate did not greatly affect the stop flow ratios of I^{131}. These experiments are interpreted as indicating passive reabsorption of iodide in both proximal and distal portions of the renal tubules of the dog.

1. Giebisch, G., MacLeod, M. B., Kavaler, F., *Am. J. Physiol.*, 1956, v187, 529.
2. Bricker, N. S., Hlad, C. J., Jr., *J. Clin. Invest.*, 1955, v34, 1057.
3. Malvin, R. L., Wilde, W. S., Sullivan, L. P., *Am. J. Physiol.*, 1958, v194, 135.
4. Kennedy, T. J., Jr., Hilton, J. G., Berliner, R. W., *ibid.*, 1952, v171, 164.
5. Smith, H. W., Finkelstein, N., Aliminosa, L., Crawford, B., Graber, M., *J. Clin. Invest.*, 1945, v24, 388.
6. Brun, A. C., *Nord. Med.*, 1949, v42, 1774.
7. Pitts, R. F., Gurd, R. S., Kessler, R. H., Hierholzer, K., *Am. J. Physiol.*, 1958, v194, 125.
8. Kessler, R. H., Hierholzer, K., Gurd, R. S., Pitts, R. F., *ibid.*, 1958, v194, 540.
9. ———, *ibid.*, 1959, v196, 1346.
10. Riggs, D. S., *Fed. Proc.*, 1949, v8, 328.
11. Halmi, N. S., King, L. T., Widner, R. R., Hass, A. C., Stuelke, R. G., *Am. J. Physiol.*, 1958, v193, 379.
12. Halmi, N. S., Stuelke, R. G., Schnell, M. D., *Endocrinology*, 1956, v58, 634.
13. Solomon, S., *J. Cell. Comp. Physiol.*, 1957, v49, 351.
14. Gilman, A., Philips, F. S., Koelle, G. S., *Am. J. Physiol.*, 1946, v146, 348.

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Comparative Stability of Trypsin and Chymotrypsin in Human Intestinal Juice. (27092)

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Intramuscular administration of trypsin and chymotrypsin is frequently resorted to in various inflammatory conditions. In view of its ease of administration, oral forms would be more desirable, providing that these enzymes are not destroyed in the gastrointestinal tract. Destruction of the proteolytic enzymes in the stomach need not be a problem, since tablets may be enterically coated so that they disintegrate in the small intestine. However, once the enzymes are released into the intestinal juice they must be stable for a sufficient length of time to permit absorption.

Pancreatic juice contains inhibitors which inactivate trypsin(1-6). This inactivation is very rapid(1,4,5) and is complete at a pH of 7 within one minute(4). The trypsin inhibitor complex is quite stable, having a dissociation constant of 2×10^{10}(4). Chymotrypsin is relatively unaffected by these inhibitors(2,3,5).

The results obtained from the study of pancreatic juice may not necessarily apply to intestinal juice because the latter is a mixture of pancreatic juice, gastric outflow, bile, and intestinal secretions. In view of the work cited above, the authors felt that a study of the stability of trypsin and chymotrypsin in complete human intestinal juice would be useful.

Materials and methods. Intestinal juice was obtained from 5 normal fasting humans by use of a Rehfuss tube. The end of the tube was positioned by fluoroscopy into the lower duodenum and pH was frequently determined during collection to insure that the tube was not withdrawn toward the stomach. In no case was the pH lower than 6.1. The aspirated juice was collected in an ice cooled container and was used within one hour after collection.

Tubes containing the weighed enzymes* were placed in a water bath at 37.5°C and 10 ml of the intestinal juice was pipetted into them under constant stirring. Solution was complete within one minute. Incubation of the intestinal juice, intestinal juice with chymotrypsin and intestinal juice with trypsin

* Trypsin and chymotrypsin used were supplied by Wampole Laboratories, Stamford, Conn.

TABLE I. pH and Enzymatic Activity of Intestinal Juice Samples.

Sample	pH	Hb units/ml*
I	6.1	12
II	7.9	23
III	7.4	26
IV	7.2	33

* The Hb unit for intestinal juice is calculated as follows: 100 × O.D. at 280 mμ. As can be seen from the method described, the added enzymes are diluted 500 times before 2 ml addition of Hb reagent while intestinal juice is diluted 50 times.

was started 3 minutes apart to permit subsequent withdrawal of aliquots at identical time intervals. One ml samples were periodically withdrawn from each tube, diluted with distilled water to 50 ml, and immediately assayed in duplicate for proteolytic activity against denatured hemoglobin by the modified Anson method(7). Two ml of this solution was added to 5 ml of denatured hemoglobin and the enzymatic reaction was allowed to proceed for 10 minutes. At the end of this period the reaction was stopped by addition of 10 ml of trichloroacetic acid and the resulting precipitate was removed by filtration. The filtrate was read at 280 mμ in a 1.0 cm cell in a Beckman D.U. spectrophotometer.

The proteolytic activity of the added enzymes is reported in terms of hemoglobin units defined as 1000 times the optical density at 280 mμ when the above dilutions are used. Since the enzymatic activity of the intestinal juice and the enzymes dissolved therein are additive (Table II), the activity of the added enzymes at the end of each period was obtained by substracting the endogenous enzymatic activity of the intestinal juice after incubation for an equal time period.

In another experiment to determine significance of pH, 450 Hb units of both trypsin and chymotrypsin were similarly incubated in U.S.P. simulated intestinal fluid(8) (pH 7.5) without the usually added pancreatin and assayed as above.

Results. The pH and initial enzymatic activity of the intestinal juice samples used in the experiments are shown in Table I. The highest enzymatic activity found for intestinal juice itself is only 33 Hb units. This is one-fifteenth the activity of the smallest quantity of added enzyme used in the experiments. The pH ranged from 6.1 to 7.9.

The results obtained from the experiments in which inactivation of trypsin and chymotrypsin in human intestinal juice were studied are presented in Fig. 1. Chymotrypsin is more stable in the intestinal juice than trypsin.

The results of the experiment in which the enzymes were incubated in an aqueous solution at pH 7.5 are shown in Fig. 2. Here again trypsin was inactivated to a greater extent than was chymotrypsin, but extent of inactivation was less than in the intestinal juice.

Discussion. Incubation of equal weights of the enzymes in intestinal juice showed that mean enzymatic activity remaining after one-

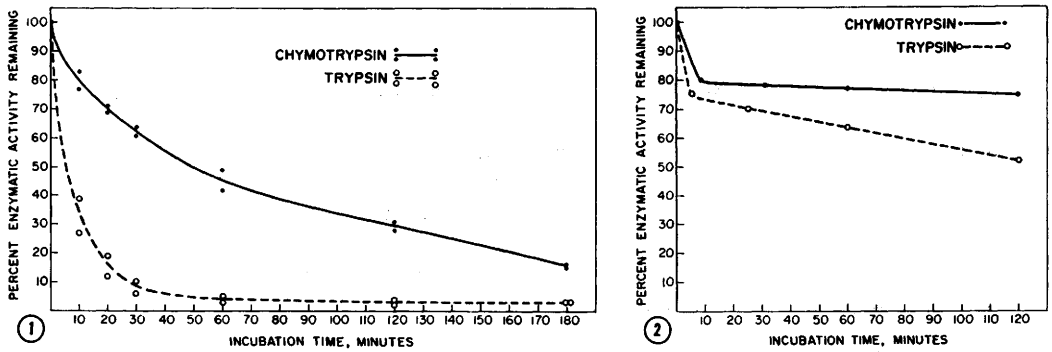

FIG. 1. Enzyme inactivation in intestinal juice. 20 mg of chymotrypsin and trypsin per 10 ml of intestinal juice.

FIG. 2. Enzyme inactivation in aqueous solution at pH 7.5. 450 Hb units of chymotrypsin and trypsin per 10 ml of USP XVI simulated intestinal juice without pancreatin.

TABLE II. Additivity of Enzymatic Activity of Intestinal Juice and Added Enzymes.

Sample	Activity as measured by optical density at 280 mμ Found	Calculated	% recovery
Exp. I			
Chymotrypsin (2 ml)	.214		
Trypsin (2 ml)	.457		
Intestinal juice (2 ml)	.288		
Chymotrypsin (1 ml) & intestinal juice (1 ml)	.233	.251	93
Trypsin (1 ml) & intestinal juice (1 ml)	.344	.373	92
Exp. II			
Chymotrypsin (2 ml)	.265		
Trypsin (2 ml)	.449		
Intestinal juice (2 ml)	.041		
Chymotrypsin (1 ml) & intestinal juice (1 ml)	.156	.153	98
Trypsin (1 ml) & intestinal juice (1 ml)	.278	.245	113
Mean recovery of 2 experiments:			
Trypsin & intestinal juice			103%
Chymotrypsin & intestinal juice			96%

half hour was 63% for chymotrypsin, and 8% for trypsin (Fig. 1).

When both enzymes were incubated in aqueous solution at pH 7.5, 79% of the chymotrypsin and 69% of the trypsin remained active after one-half hour (Fig. 2). Since the relatively rapid inactivation of trypsin cannot be attributed to the pH of the intestinal juice alone, these results suggest that trypsin inhibitors(2,3,5) from the pancreatic juice are still active in the intestine. It is also possible that the enzyme proteins are destroyed by proteolytic activity *per se*.

Summary. The stability of trypsin and chymotrypsin was determined in human intestinal juice. On a weight per weight basis at the end of one-half hour as much as 63% of chymotrypsin was still enzymatically active while only 8% of the trypsin activity could be detected. In fact, 30% of the chymotrypsin still remained at the end of 2 hours.

1. Haverback, B. J., Dyce, B., Bundy, H., Edmondson, H. A., *Am. J. Med.*, 1960, v29, 424.

2. Wu, F. C., Laskowski, M., *J. Biol. Chem.*, 1955, v213, 609.

3. Grossman, M. I., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 304.

4. Green, N. M., Work, E., *Biochem. J.*, 1953, v54, 347.

5. Northrop, J. H., Kunitz, M., Herriott, R. M., *Crystalline Enzymes*, 1948, 2nd ed., p153, Columbia Univ. Press, N. Y.

6. Shylgin, G. K., *Byull, Eksptl. Biol. Med.*, 1941, v11, 456. (*Chem. Abstr.* v41, 6294ª).

7. Ref. 5., pp303-305.

8. *Pharmacopeia of U.S.A.*, 1960, 16th Revision, p1073, Mack Publishing Co., Easton.

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Glycyrrhetinic Acid on Female Response to Exogenous and Endogenous Sex Steroids.* (27093)

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The anti-estrogenic activity of beta glycyrrhetinic acid in immature intact as well as adrenalectomized rats has been reported(1). The previous study(1) was concerned with the restriction of the uterotrophic response to estradiol benzoate in immature rats with intact gonads. This investigation was undertaken to determine whether the antagonism was limited to the uterine response to exogenous estrogens and whether its effect was dependent upon the presence of gonads or pituitary.

Methods and materials. Rats of the Wistar strain were maintained under constant temperature (25°C) and lighting (12 hours light) and were fed a Rockland rat diet and water *ad libitum*. Hypophysectomized rats received 24 hours after surgery were

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