ibid., 1958, v63, 570.

3. Urist, M. R., *Rec. Adv. in Hormone Res.*, Acad. Press Inc., New York, 1959, v15, 455.

4. Jones, I. C., *The Adrenal Cortex,* Cambridge Univ. Press, 1957.

5. Hublé, J., *Natur. schop. Tijdschr.*, 1957, v39.

6. Brown, K. I., Meyer, R. K., Brown, D. J., *Poult. Sci.*, 1958, v37, 680.

7. DiRaimondo, V. C., Forsham, F. H., *Metab. Clin. Exp.*, 1958, v7, 5.

8. Nelson, D. H., Samuels, L. T., *J. Clin. Endocrinol.*, 1952, v12, 519.

9. Silber, R. H., Bush, R. D., Oslapas, R., *Clin. Chem.*, 1958, v4, 278.

10. Sturkie, P. D., *Avian Physiology,* Comstock Publ. Assn., Ithaca, N. Y., 1954.

11. Phillips, J. G., Jones, I. C., *J. Endocrinol*, 1958, v16, iii.

12. Riddle, O., Honeywell, H. E., *Am. J. Physiol*, 1923, v66, 340.

13. Riddle, O., Reinhart, W. H., *ibid.*, 1926, v76, 660.

14. Riddle, O., Burns, F. H., *ibid.*, 1927, v81, 711.

15. Perek, M., Eckstein, B., *Poult. Sci.*, 1959, v38, 996.

16. Storey, E., *Austral. and New Zeal. J. Surg.*, 1957, v27, 19.

17. Francis, D. W., *Poult. Sci.*, 1957, v36, 181.

18. Bell, D. J., Siller, W. G., Campbell, J. G., *J. Biochem., Proc. Biochem. Soc.*, 1959, v72, 32p.

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Erythropoietic Activity of Proteolytic Enzymes. (25719)

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During some studies on effect of certain enzymes on activity of erythropoietin preparations, we found that samples of papain and bromelin could stimulate erythropoiesis. Consequently, a number of other proteolytic enzymes were assayed.

Methods and materials. A modification of the methods of Fried *et al.*(1) and Rambach *et al.*(2) was used to measure erythropoiesis. Each sample was injected subcutaneously daily for 4 days into 6 female Holtzman rats. Food was withheld throughout experiment but water was given *ad lib.* On fourth day, 0.5 μc of Fe^{59}, as ferric citrate, was injected into the tail vein. Twenty-four hours later amount of radioactive iron in an aliquot of whole blood was measured. In calculating % of Fe^{59} uptake by total red cell mass, it was assumed that blood volume was 5.18% of body weight at time of sacrifice. *Enzymes* were obtained from commercial companies (Table I). Sialic acid was determined by direct Ehrlich reaction described by Werner and Odin(3).

TABLE I. Erythropoietic Activity of Proteolytic Enzymes.

Enzyme	Purity	Source	Dose, mg	Fe^{59} uptake, avg %
Pepsin	Crystalline	Worthington	.5	7.4 ± .9
Trypsin	"	"	.1	11.1 ± 1.6
Chymotrypsin	"	"	.1	11.2 ± .2
B. subtilis proteinase	Crude	Novo	.5	9.2 ± 1.1
Bromelin	"	Takamine	.5	13.6 ± 1.1
Ficin	"	Lilly	.5	9.1 ± 1.4
Control				10.3 ± .9
Papain	Crude extract	Penick	5.0	14.3 ± 1.2
Control				7.8 ± 1.0
Papain	Partially purified	Lilly	.2	13.1 ± 1.3
Control				10.1 ± 1.5
Papain	Crystalline	Worthington	.1	13.4 ± 1.5
Control				10.3 ± .9

Results. Erythropoietic activity was detected in crude preparation of bromelin and in crude, partially purified, and crystalline preparations of papain. Other proteolytic enzymes showed no activity. The data are summarized in Table I.

Crystalline papain contained 1.1% of sialic acid.

Discussion. Our results raise the following questions: (1) Is erythropoietic activity of papain related to its proteolytic activity or to a specific structure of the enzyme molecule? (2) Is sialic acid an impurity in crystalline papain preparations or is it part of the enzyme molecule? These require further investigation.

Summary. Crystalline papain and crude bromelin preparations stimulate erythropoiesis, whereas other proteolytic enzymes are ineffective. Sialic acid, measured by direct Ehrlich reaction, has been detected in crystalline papain preparations.

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1. Fried, W., Plzak, L. F., Jacobson, L. O., Goldwasser, E., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 237.

2. Rambach, W. A., Alt, H. L., Cooper, J. A. D., *Blood*, 1957, v12, 1101.

3. Werner, I., Odin, L., *Acta Soc. Med. Upsalien*, 1952, v57, 230.

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Thyroid Hormone Binding Proteins in Human Serum Characterized by Immuno-Electrophoresis and Auto-Radiographic Tracings.* (25720)

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There is no general agreement on the nature of linkage of thyroxin and triiodothyronine to human serum proteins(1). Identification, isolation and investigation of these binding proteins are essential to understanding the transport of thyroid hormone and may involve a better knowledge of metabolism of the hormone. By a combination of immuno-electrophoresis of human sera incubated with radio-thyroxin and radio-triiodothyronine and autoradiographic technic it has been possible to identify exactly the thyroxin- and triiodothyronine-binding proteins as the same 3 lipoproteins. This combination of immuno-electrophoresis with tracer technic seems to be the method for identifying and estimating linkage of organic and inorganic compounds to serum proteins.

Methods. Immuno-electrophoresis(2) was performed as described by Scheidegger(3) at pH 8.6 and stained for serum proteins. Lipoproteins were demonstrated with Oil red O (4). Ten to 20 μl of human serum were incubated with corresponding volume of a radio-L-thyroxine or radio-L-triiodothyronine solution in 50% propylene-glycol (Abbott Laboratories). Activity was 2-3 μc/sample and by incubation about 0.3 10^{-9} M thyroxin or 0.2 10^{-9} M triiodothyronine were added to original hormone content of serum. After immuno-electrophoresis the slides were placed in direct contact with Kodak X-ray film or Kodak autoradiographic film for a week and then developed.

Results. Fig. 1 shows immuno-electrophoresis of normal serum incubated with thyroxine (upper half) and triiodothyronine (lower half) and stained conventionally for serum protein precipitation bows with amido-black. Fig. 2 shows corresponding autoradiographs. Only 3 different precipitation bows contain radioactivity. From their mobility these 3 thyroxine- and triiodothyronine-binding proteins must be identified with the 3 known lipoproteins in normal human serum, *i.e.*, alpha-2-lipoprotein (the slow moving lipoprotein fraction), alpha-1-lipoprotein (the

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