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)

J. CLAUSEN AND T. MUNKNER (Introduced by G. Asboe-Hansen)

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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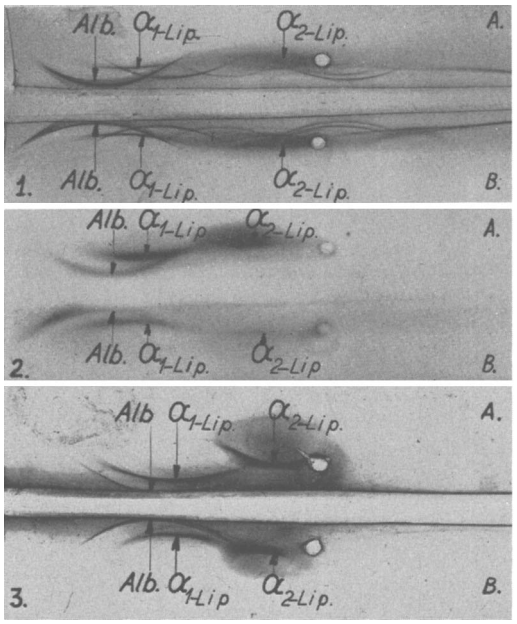

FIG. 1. Photo of immuno-electrophoresis colored conventionally for serum protein precipitation bows with amidoblack. A. Immuno-electrophoresis of normal human serum incubated with thyroxine. B. *Idem* with triiodothyronine.

FIG. 2. Photo of same tracings as in Fig. 1 developed as autoradiogram.

FIG. 3. Photo of immuno-electrophoresis of same sera as in Fig. 1 colored for lipoproteins with Oil red O.

faster moving lipoprotein, the mobility of which may vary to some extent and range from prealbumin area to the cathodical part of the alpha-1-area, depending on age of serum), and lipalbumin. Fig. 3 shows immuno-electrophoretical tracings of sera stained for lipoproteins with Oil red O, autoradiographic blackening exactly corresponding with the 3 lipoproteins.

Discussion. Previous studies concerning the possible existence in serum of a specific thyroxine- or triiodothyronine-binding protein have been confusing, because conventional methods for separating serum proteins do not correlate, giving fractions with different properties. *Salting out procedures* give no sharp zone of precipitation for identification of such a protein. *Moving-boundary electrophoresis* (5), too, reveals a varying iodination of the different protein fractions. Robbins & Rall (6) and Horst & Rösler(7) using *conventional paper-electrophoresis* found that thyroxine was bound to a protein fraction in the area between alpha-1 and alpha-2 globulins and to a smaller extent to a fraction in the albumin area. *Acid electrophoresis and ultracentrifugation* have suggested thyroxine-binding protein to be an alpha-2- glycoprotein with a sedimentation rate $S_{20,w}$ of 3.3(8) and (9). *Cohn fractionation*(10,11) seems to indicate that the protein is localized to fraction IV, V and possibly to VI. Fractions IV and V mainly contain albumin, alpha- and beta-globulins(12). Finally Rich and Bearn (13) using *starch gel electrophoresis* found a high specific thyroxine binding capacity in the prealbumin area.

In immuno-electrophoresis only the antigen-antibody precipitate corresponding to each protein fraction in serum is left, whereas any excess of antigen or antibody is removed by repeated washings with saline. Hence only protein linked thyroxine and triiodothyronine involved in the precipitate will remain on the slide, while all free hormone, all thyroxine or triiodothyronine which was only loosely bound to the precipitate, and any complexes between hormone and nonreacting proteins will not be retained in the immuno-electrophoresis. We therefore conclude that only the 3 lipoproteins in human serum have the capacity to bind thyroid hormone in serum. We could not confirm a specific and very firm binding between any carbohydrate-containing alpha-serum protein (for example Schultze's alpha-2-macroglobulin or the alpha-1-glycoprotein(14)). The results obtained by other authors using more conventional fractionation methods can be explained by immuno-electrophoretical findings in prealbumin, albumin, and alpha areas. Immuno-electrophoresis can unveil 3 different well known serum proteins in the alpha-1 area (*i.e.*, alpha-1-lipoprotein, alpha-1-glycoprotein and alpha-1-seromucoid) with almost the same mobility, whereas these proteins cannot be separated by paper electrophoresis. Next, mobility of alpha-1-lipoprotein is variable (15) depending on how long the serum has been stored. Fresh alpha-1-lipoprotein shows an alpha-1-mobility, whereas serum stored for some time appears with some splitting of lipoprotein, giving rise to a new precipitation

bow with prealbumin mobility(15), so that the sum of the amount of alpha-1-lipoprotein and lipo-prealbumin remains constant. The complex of alpha-1-lipoprotein and radio-thyroxine or radio-triiodothyronine on auto-radiograph may therefore show some variability as a function of storing and possibly, by chemically induced splitting by addition of the labelled hormone. Also the lipoprotein with alpha-2-mobility seems to have a somewhat variable position depending on amount of lipo- and normal serum proteins(15). Thus on the basis of immuno-electrophoretical tracings within the lipoprotein system and our investigation of binding properties, the confusing results which have been obtained concerning thyroxine binding proteins in human serum are understandable. The reason some workers have found a very high thyroxine binding capacity to the tryptophan rich prealbumin of Schultze(16), is being investigated, as our antisera have a very low titer against this protein.

Summary. Immuno-electrophoresis of normal human serum incubated with radio-thyroxin and radio-triiodothyronine combined with auto-radiography and special staining for lipoproteins with Oil red O showed that the albumin (identical with lipalbumin), alpha-1-lipoprotein and alpha-2-lipoprotein have the capacity to bind thyroxin and triiodothyronine.

1. Pitt-Rivers, R., Tata, J. R., *The thyroid hormones.* Pergamon Press, London, 1959.
2. Grabar, P., Williams, C. A., *Biochem. biophys. Acta,* 1953, v10, 193.
3. Scheidegger, J. J., *Internat. Arch. Allergy,* 1955, v7, 103.
4. Schwick, G., *Laboratoriums-Blätter,* 1958, v1, 11.
5. Silver, S., Reiner, M., *Bull. N. Y. Acad. Med.,* 1950, v26, 277.
6. Robbins, J., Rall, J. E., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 530.
7. Horst, W., Rösler, S. R., *Klin. Wschr.,* 1953, v31, 13.
8. Robbins, J., Petermann, M. L., Rall, J. E., *J. Biol. Chem.,* 1955, v212, 403.
9. Petermann, M. L., Robbins, J., Hamilton, M. G., *ibid.,* 1954, v208, 369.
10. Freinkel, N., Dowling, J. T., Ingbar, S. H., *J. Clin. Invest.,* 1955, v34, 1698.
11. Schmidt, K., *J. Am. Chem. Soc.,* 1953, v75, 60.
12. Cohn, E. J., Strong, L. E., Hughes, W. L., Jr., Mulford, D. J., Ashworth, J. N., Melin, M., Taylor, A. L., *ibid.,* 1946, v68, 459.
13. Rich, C., Bearn, A. G., *Endocrinol.,* 1958, v62, 687.
14. Schultze, H. E., Göllner, I., Heide, K., Schönenberger, M., Schwick, G., *Naturf.,* 1955, v10B, 463.
15. Uriel, J., *Clin. Chim. Acta,* 1958, v3, 384.
16. Schultze, H. E., Schönenberger, M., Schwick, G., *Biochem. Z.,* 1956, v328, 267.

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Effect of *Pseudomonas* on Lactate Production by Mouse Monocytes.* (25721)

R. S. BERK AND E. L. NELSON (Introduced by A. J. Salle)
Dept. of Bacteriology, University of California, Los Angeles

Recent data obtained in this laboratory indicate that succinoxidase activity of cells obtained from mouse peritoneal exudates, as well as of homogenates of liver and spleen, was inhibited in presence of heat-killed *Pseudomonas aeruginosa*(1). However, since metabolism of mouse monocytes appears to be mainly glycolytic, this study was undertaken to determine in what way, if any, *P. aeruginosa* might affect lactate production by these cells.

Method. Warm mineral oil (0.5 ml/mouse) was used to induce exudates in 10 week old female mice (Webster strain), 18 hours before harvesting of cells from peritoneal cavities with chilled 0.9% sodium chloride containing 0.1% heparin. Pooled monocytes were washed once with saline by centrifuging for 10 minutes at 500 x G. In experiments with

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